THE UNIVERSITY
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Farming Practices and Trypanosomiasis in Northern Uganda

An Assessment of Trypanosomiasis Prevalence and the Ongoing Management of Vector Borne Infections

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Declaration

I declare that this thesis is my own composition, the research described within this thesis is my own work and that this work has not been submitted for any other degree or professional qualification.

Liam D Miller

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Abstract

African trypanosomiasis is a parasitic infection caused by a number of species of the genus Trypanosoma. The disease, in various forms, affects wildlife, livestock and humans and is transmitted by the bite of the tsetse fly. There are two forms of human African trypanosomiasis, each caused by a different species of Trypanosoma; the acute form (rHAT) and the chronic form (gHAT). Overlap of the two forms would complicate treatment. Trypanosomiasis in cattle is known as African animal trypanosomiasis (AAT) and the effects of AAT cause significant economic damage as meat and milk production are reduced and cattle become too weak to pull ploughs. Cattle can also carry T. b. rhodesiense. Uganda is affected by both rHAT and gHAT but the diseases do not occur in the same area of the country however the distance between the rHAT and gHAT-areas has decreased in recent years.

This study investigates the prevalence of AAT in northern Uganda and the ways in which farmers are attempting to control the disease in their cattle.

Prevalence of AAT in the study district was found to be low, with only 2.61% cattle infected. Local breeds of cattle were to be less likely to be infected than European breeds. Farmers in the area are generally not treating their animals for AAT and are not spraying their cattle with insecticides that kill tsetse flies.

The AAT situation in northern Uganda is currently stable but there are a number of current and future developments that threaten the status quo. Increased prevalence of AAT in cattle and overlap of areas affected by the two forms of HAT could have severe impacts on the economic security and health of the rural population of Uganda. Monitoring the evolving situation is of great importance.

Lay Summary

African trypanosomiasis is a parasitic infection caused by a number of species of the genus Trypanosoma. The disease, in various forms, affects wildlife, livestock and humans and is transmitted by the bite of the tsetse fly. There are two forms of human African trypanosomiasis, each caused by a different species of Trypanosoma; the acute form (rHAT) and the chronic form (gHAT). Overlap of the two forms would complicate treatment. Trypanosomiasis in cattle is known as African animal trypanosomiasis (AAT) and the effects of AAT cause significant economic damage as meat and milk production are reduced and cattle become too weak to pull ploughs. Cattle can also carry T. b. rhodesiense. Uganda is affected by both rHAT and gHAT but the diseases do not occur in the same area of the country however the distance between the rHAT and gHAT-areas has decreased in recent years.

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1. Introduction

This introductory chapter will cover a general background of trypanosomiasis in animals and humans, its management and will situate this study within the current body of work regarding the epidemiology of *T. brucei* s.l. and *T. b. rhodesiense* in Uganda.

1.1. Trypanosomiasis

The trypanosomiases are a group of infectious diseases occurring in humans and animals caused by various species of the protozoal parasites of the genus *Trypanosoma.* The human infective trypanosomes are *T. b. rhodesiense* and *T. b. gambiense*, each causing a different form of Human African Trypanosomiasis (HAT), also known as sleeping sickness, both of which are debilitating and, if untreated, fatal in the vast majority of cases. There are more species of trypanosome capable of causing African Animal Trypanosomiasis (AAT) or nagana, namely, *T. vivax, T. godfreyi, T. simiae, T. b. brucei* and *T. congolense.*

American trypanosomiasis or Chagas disease is caused by *Trypanosoma cruzi* and occurs in South America. However this study will only concern the African trypanosomiases, AAT and HAT, as *T. cruzi* is a distant member of the genus *Trypanosoma*, as shown in Fig. 1.

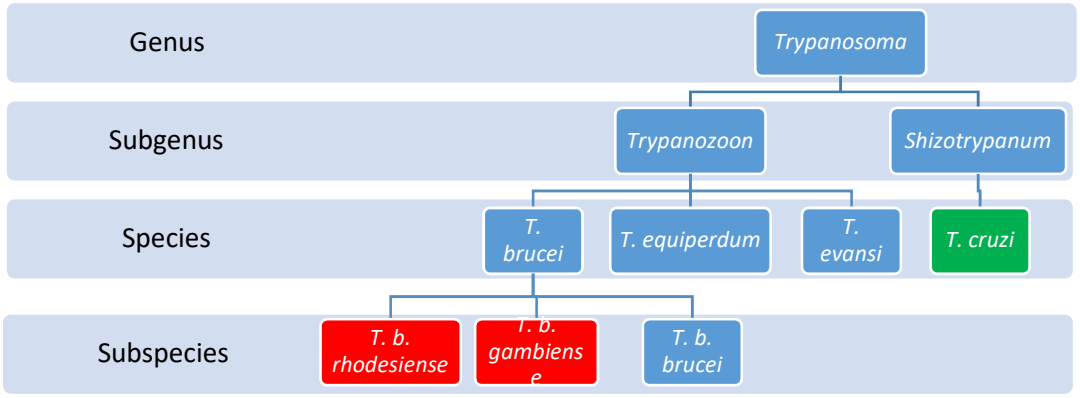

Figure 1. Phylogenetic tree showing relationship between the causative organisms of human African trypanosomiasis (red) and American trypanosomiasis (green)

1.2. Tsetse fly

The African trypanosomes are vector-borne parasites occurring in sub-Saharan Africa in those areas inhabited by the tsetse fly (genus *Glossina*). The *Glossina* genus contains thirty-one species and subspecies, separated into three groups with differing habitats: the palpalis, morsitans and fusca. Although all species of tsetse are capable of serving as vector for trypanosomes, *G. f. fuscipes* is the principal vector of trypanosomiasis in Uganda. Waiswa et al (2006) found that 99.9% of flies captured in south-eastern Uganda were *G. f. fuscipes.*

Transmission of trypanosomes by tsetse is cyclical as the parasite undergoes stages of its lifecycle at different sites inside the tsetse fly; infection occurs following ingestion of bloodstream trypomastigotes within a blood meal taken from an infected host. Bloodstream trypomastigotes transform into procyclic trypomastigotes in the tsetse midgut where they proliferate by binary fission. The procyclic trypanosomes migrate to the salivary glands of the tsetse where they first transform into epimastigotes that undergo further binary fission before transforming into metacyclic trypomastigotes. The time period from ingestion of bloodstream trypomastigotes to establishment of infection within the tsetse fly and transformation to metacyclic trypomastigotes lasts approximately 3 weeks (CDC, 2015), after which the tsetse fly is able to infective.

It is in this form that onward transmission will occur as trypanosomes are inoculated into the susceptive animal host when the tsetse fly takes a bloodmeal. Once in the blood of the animal host, metacyclic trypomastigotes transform into bloodstream trypomastigotes which proliferate by binary fission in the blood, lymph and, in some instances, the cerebrospinal fluid (CSF) (CDC, 2015). Fig. 2. Illustrates the changing morphology of trypanosomes throughout the lifecycle and the locations in which they take on these forms (Pays et al, 2006).

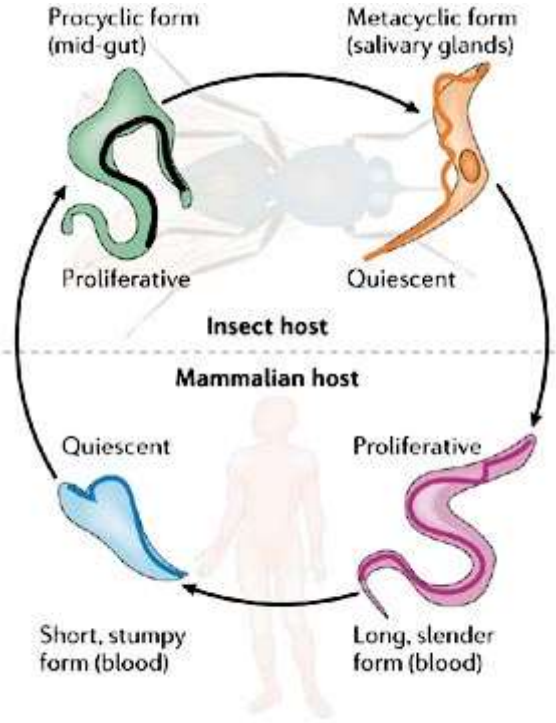

Figure 2. The stages of the trypanosome life cycle. Reproduced from Lee et al (2007).

Tsetse species are K-strategists which means that they produce few progeny with a high rate of survival, as opposed to R-strategists that produce a large number of progeny with a low rate of survival (Leak, 1998). This is uncommon among *Diptera* and insects as a whole and is the result of peculiar life cycle and reproductive method of the species. Tsetse are larviparous, meaning that the female fly nurtures a single larva within its uterus and provides nutrition through secretions of a specialised milk gland. The female then deposits a third instar larva which will dig into the ground where it becomes a puparium for a period of thirty to forty days before emerging as a developed adult fly. Female tsetse in experimental colonies produce around ten offspring during their adult life (Leak, 1998). As a result of this low reproductive rate, trypanosomiasis control methods targeting the tsetse vector are an effective means of interrupting parasite transmission.

1.3. Human African Trypanosomiasis

Human African Trypanosomiasis, as previously stated, exists in two forms caused by *T. b. gambiense* (gHAT) and *T. b. rhodesiense* (rHAT). Infection by *T. b. gambiense* causes the chronic form of the disease, which make up 98% of all reported cases of HAT (Franco et al, 2014). Cases of gHAT are found in western and central Africa with the majority of cases occurring in the Democratic Republic of the Congo (DRC) (Simarro et al. 2010). There is a focus of the disease in north-western Uganda and this is the eastern extent of the disease distribution.

T. b. rhodesiense HAT is zoonotic whereas the main reservoir of *T. b. gambiense* sleeping sickness is infected human hosts. The animal reservoir of *T. b. rhodesiense* is in wildlife and, particularly in Uganda, livestock including cattle and pigs. This results in a very different epidemiology of the two forms of disease.

T. b. gambiense and *T. b. rhodesiense* are able to infect humans due to genes found only in these subspecies that confer resistant to human serum. The SRA (Serum Resistance Associated) gene in *T. rhodesiense* (Xong et al, 1998) and the TgsGP gene in *T. b. gambiense* (Capewell et al, 2013) confer this resistance and are sub-species specific.

The course of both forms of HAT can be separated into two stages. The first haemolymphatic stage is the period when trypanosomes inhabit the tissues outside of the central nervous system (CNS) e.g. blood and lymph. During this stage of HAT the signs and symptoms include development of a chancre at the site of inoculation, headaches, fever, Winterbottom's sign (posterior cervical lymphadenopathy) and weakness, among others. This early stage generally lasts for a period of around one and a half years in gHAT before

trypanosomes cross the blood-brain barrier and enter the CNS (Checchi et al, 2008a). The second meningoencephalitic stage presents with an array of neurological symptoms including irritability, changes in personality and behaviour, and lassitude and as the infection progresses further, delirium, hallucinations, and uncontrolled sexual impulses. (Pentreath and Kennedy, 2004). A cardinal symptom of the meningoencephalitic stage of gHAT is progressively worsening somnolescence, this being the origin of the name 'sleeping sickness'. If untreated, the patient progresses to a state of constant sleep before death. This usually occurs within three years of infection in gHAT, although recent work has found that not all untreated cases are fatal (Jamonneau et al. 2012), with individuals becoming 'silent carriers' that may maintain infection at undetectably low levels of parasitaemia (Welburn, et al 2016).

Infection by *T. b. rhodesiense* causes the acute form of HAT. This form of the disease is found in East and Southeast Africa (Simarro et al. 2010). Historically, the disease has existed in the south-eastern region of Uganda, south of Lake Kyoga but in recent years the distribution of the pathogen has expanded to the north west. The progression of rHAT is much faster than that of the gHAT and more than 80% of untreated cases die within six months of infection (Odiit et al, 1998). Organ failure (e.g. heart) is often the cause of death in rHAT cases; perimyocarditis is important in the fatal outcomes of the disease (Kuepfer et al, 2011).

In addition to morbidity and mortality caused by HAT, the disease economically harms the dependents of the sufferer. Accurate diagnosis of HAT is often not made on the first visit to healthcare facilities. Odiit et al (2004) found that in a sample of HAT patients in Tororo district, only 11.8% (14/119) were correctly diagnosed on their first visit to a healthcare facility and the most common visit where a correct diagnosis was made was the third, with 33.6% (40/116) of cases. For 16% (19/116) of sufferers, more than four visits were required before the correct diagnosis was made. Odiit et al found that most patients were misdiagnosed as having malaria on their first presentation at a healthcare facility.

This misdiagnosis may be due in part to the similarities between the clinical presentation of early stage HAT and malaria (Farrar, 2013). The use of rapid diagnostic tests (RDTs) in the diagnosis of malaria and HIV, two infections endemic in Uganda, is on the increase. This is leading to the substitution of microscopy in malaria diagnosis, owing to the accuracy of the RDTs. The replacement of microscopy removes the possibility of serendipitous detection of trypanosomes in a sufferer under investigation for malaria (Lejon et al, 2013). The sensitivity and specificity of HIV RDTs exceeds 98% but in HAT-infected individuals this can fall to

39% (Lejon et al, 2010). Thus the risk of false-positive diagnosis of HIV and the reduced chances of detecting trypanosomes during microscopy screening for malaria decrease the likelihood of an accurate HAT diagnosis being made.

With repeated visits to potentially distant healthcare facilities and incorrect diagnoses come costs of travel and of inappropriate treatments that can amount to a serious financial drain on the families of those affected.

As well as costs incurred due to the management of HAT, the loss of ability to work further impacts of household finances. Odiit (2004) found that 67.2% (80/119) of sufferers were between the ages of 15 and 54 and that 92.4% (110/119) of the sufferers were full time farmers by occupation. For smallholder farmers, inability to work seriously impacts upon income and as individuals of working age are the most commonly affected, HAT threatens economic security of rural households affected.

1.3.1. Diagnosis of Human African Trypanosomiasis

Due to the toxicity of the chemotherapeutic agents used in treatment of HAT, definitive diagnosis must be made before commencement of treatment. Despite the advent of molecular techniques and development of species-specific PCR assays, the use of these diagnostic methods is largely restricted to research settings as due to logistic and equipment requirements, use at the point of care is not felicitous. The areas affected by sleeping sickness are resource-poor and rural and as a result, mobile surveillance teams and health centres use diagnostic techniques with lower requirements of equipment, reagents and staff training.

Owing to its simplicity, microscopy of lymph node aspirate or blood film is usually the first diagnostic test performed (Deborggraeve and Büscher, 2010). This technique is more sensitive in cases of *T. b. rhodesiense* HAT, as due to high levels of parasitaemia, trypanosomes should be identifiable on a thin or thick blood smear (Kennedy, 2013). As the parasitaemia is lower and fluctuates in *T. b. gambiense* infection, this species may not be detected through microscopy. However, mobile surveillance teams and healthcare facilities operating in the gHAT foci have the Card Agglutination Test for Trypanosomiasis (CATT) at their disposal. This cheap and highly serological diagnostic is an agglutination assay that detects serum antibodies to the LiTat 1.3 antigen, expressed by *T. b. gambiense.* As a result, this test is of use in diagnosing gHAT infection but not rHAT. (Chappuis et al, 2005)

An important aspect of HAT diagnosis is staging of the disease. As the treatments for late stage disease are more toxic than those for the early stage due to their efficiency in crossing

the blood-brain barrier, the stage of disease must be ascertained in order to avoid unnecessary administration of these drugs, minimising iatrogenic harm. Examination of CSF is performed to determine stage of disease following a positive diagnosis of HAT. The criteria for a late stage diagnosis, as outlined by WHO (1998) are the detection of trypanosomes and/or a white cell count of ≥ 6 /μl in the cerebrospinal fluid (CSF) opposed to a normal CSF white blood cell count of ≤ 5/μl.

1.3.2. Treatment of Human African Trypanosomiasis

The treatment of HAT involves the administration of chemotherapeutic agents. Four drug treatment regimens are currently registered with the WHO and the early stage treatments differ for the two forms of the disease. In early stage rHAT, the treatment is suramin and is administers intravenously as follows: test dose of 4-5mg/kg body weight on day 1, followed by 20mg/kg doses on days 3, 10, 17, 24 and 31. The drug used in the treatment of early stage gHAT is pentamidine, administered via intravenous infusion of 4mg/kg every 24 hours for 7 days (Farrar et al, 2013). These drugs both have common adverse effects including pyrexia and mild nephrotoxicity caused by suramin and hypotension caused by pentamidine.

The front line treatment of the late stage of *T. b. gambiense* HAT is nifurtimox-eflornithine combination therapy (NECT), introduced in 2009. This combination therapy consists of short intravenous infusion of 200mg/kg of IV eflornithine every 12 hours for 7 days and 5mg/kg of oral nifurtimox every 8 hours for 10 days. This regime is an improvement on eflornithine monotherapy as it simplifies, shortens, and reduces staff and logistical requirements of treatment (Malvy and Chappuis, 2011) and is less toxic than melarsoprol (Farrar et al, 2013).

Preceding the advent of NECT, the only drug used in the treatment of late stage HAT was melarsoprol, and this remains the front-line treatment for late stage *T. b. rhodesiense* HAT as the pathogen is innately resistant to eflornithine. There are significant problems with adverse effects of this drug. The most severe and life-threatening of these is the development of a reactive encephalopathic syndrome which causes death in 3-6% of patients receiving the drug. (Chappuis, 2007) Melarsoprol is administered intravenously as 2.2mg/kg every 24 hours for 10 days (Farrar et al, 2013).

1.4. African Animal Trypanosomiasis

AAT presents a challenge to animal health that has severe negative effects on the rural economies of sub-Saharan Africa. The disease affects many species of wildlife and livestock but AAT in cattle will be the focus of this project.

Different species of trypanosome have varying pathogenicity in livestock. *T. congolense* and *T. vivax* cause severe pathology whereas *T. b. brucei* and *T. evansi* are milder in their effect. The most prominent feature of AAT is anaemia which occurs alongside patent weakness, lethargy and loss of condition. Death may occur within weeks or months of infection or the disease may enter a chronic phase of which wasting and infertility are characteristic (Taylor and Authie, 2004). This chronic phase most often results in death after a period of months or years.

As well as losses of cattle due to death, AAT causes reduced meat and milk production and reduced manure and draught power output. This significantly harms the yield achievable in smallholder mixed crop-livestock production systems (Kristjanson et al. 1999, Swallow, 2000). Fertility is also negatively impacted, with abortions common.

As well as economic losses consequent to reduced livestock outputs, it is estimated that $35 million is spent annually by smallholder farmers on trypanocidal drugs for the treatment of AAT across Africa (Holmes et al, 2004). As a result of the effects of the disease and the costs to farmers of controlling the disease, HAT perpetuates poverty in the affected rural, poor communities.

Beyond the economic effects of disease in livestock, AAT poses a significant public health challenge as cattle are an important reservoir of zoonotic *T. b. rhodesiense*, therefore infected cattle pose an infection risk to humans and rHAT epidemics have been found to be caused by introduction of infected cattle into previously unaffected areas (Fèvre et al, 2001).

1.5. Molecular Screening of Blood Specimens

Direct diagnosis of trypanosomal infections is usually achieved either by the observation of clinical signs, or by microscopy. Both approaches are compatible with a low resource setting but lack the sensitivity and specificity required for accurate diagnosis of sub-clinical infections or determination of the infective species.

It can be argued that accuracy is not necessary in the absence of specific treatment options for individual species of trypanosomes – as is the case in livestock; we will discuss later the generic options available. In terms of appreciating the epidemiology of infection within the animal reservoir, and the potential capacity within various livestock systems to act as carries for zoonotic infections, more accurate methodologies are required. This need can be met by molecular methodologies, primarily the use of PCR to amplify the genetic materials for parasitic infections within the vertebrate host.

Laboratory analysis of blood specimens collected in the field is used to determine infection status of the sample human/animal. Individual animal infection status data can be used e.g. in risk factor analysis estimates of prevalence within a group of potential hosts can be produced. Species-specific PCR primers have been developed as well primers complementary to DNA sequences conserved across a number of species.

1.5.1. TBR-PCR

The primers for this PCR were developed by Moser et al (1989). This PCR amplifies a 177bp DNA satellite repeat sequence of which there are ten thousand copies in the genome of *T. brucei* s.l. A positive result from TBR-PCR indicates infection with either *T. b. brucei, T. b. rhodesiense, T. b. gambiense, T. evansi* or *T. equiperdum.* Moser et al (1989) were able to detect 0.1pg of DNA; the amount of present in a single trypanosome.

1.5.2. ITS-PCR

This PCR assay developed by Njiru et al (2005) uses primers complementary to the Internal Transcribed Spacer 1 region of trypanosome rDNA of which there are 200 copies/genome. This locus is a suitable target in the detection of trypanosomes as it is highly conserved and varies in length between trypanosome species (Desquesnes et al, 2001). The trypanosome species that can be detected by ITS-PCR and the size of the PCR product for each are listed in Table 1.

Species	*Size of PCR product (base pairs)*
T. vivax	250
T. godfreyi	280
T. simiae Tsavo	370
T. simiae	400
T. brucei s.l.	480
T. congolense Kilifi	620
T. congolense Savannah/Forest	700

Table 1. . Size of PCR products from ITS-PCR reaction

1.5.3. SRA-PCR

SRA-PCR is a multiplex reaction that targets both the Serum Resistance Associated (SRA) gene and the GPI-PLC gene. (Picozzi et. al., 2008)The SRA gene confers human infectivity specifically to *T. b. rhodesiense* and is therefore used as a diagnostic marker; amplification of this gene indicates that the specimen contains *T. b. rhodesiense* genetic material. The reaction contains an internal control with inclusion of primers complementary to the GPI-

PLC gene. Amplification of this sequence indicates that there is sufficient *T. brucei s.l.* genomic material to detect a single copy gene.

A multiplex PCR reaction with SRA primers and GPI-PLC primers overcomes the problem of diagnostic PCR whereby a negative result may either indicate the absence of the target organism or an insufficient amount of genomic DNA for amplification. An amplification of the GPI-PLC sequence and a lack of amplification of the SRA gene indicates presence of *T. brucei* s.l. and a definite absence of *T. b. rhodesiense.*

1.5.4. Heterogeneity of Trypanosome Genetic Material on FTA Cards as a Limitation of PCR Screening

When blood specimens are collected and applied to Whatman® FTA® cards there are issues related to the homogeneity of material distributed across the surface matrix. Cox et al (2010) investigated by isolating between 92 and 114 discs from 35 cattle blood samples on FTA cards, each disc was screened for trypanosome infection using ITS-PCR. They found that there was a significant element of stochasticity involved in detecting trypanosome infection from a single punch of a blood sample. With an increasing number of PCR screenings of each sample, the cumulative infection rate of any trypanosome species increased from an average of 9.7% when screening a single punch to 85.7% total cumulative prevalence. Screening multiple punches increases the likelihood of screening a region of the card containing trypanosome DNA, reducing the effect of the heterogeneity of genetic material within the blood on the card. This study demonstrates that screening a single disc punched from an FTA card is not suitable for making estimates of prevalence and that multiple discs must be screened.

Ahmed et al (2011) also investigated the effect of localisation of trypanosome DNA within blood samples on Whatman ® FTA® cards. They screened samples using TBR-PCR and found that screening an increased number of discs taken from a transect across the surface increased sensitivity. Significant increases in sensitivity were found when using ten 0.2mm discs compared to using five or less.

Ideally, the whole of the sample could be screened as this would ensure maximum sensitivity of the PCR screening but using more discs from each card will deplete a library of specimens. This will prevent the use of samples for further analysis in future studies, and as field sample collection exercises are labour, time and resource intensive, this must be avoided. In order to compromise between unsatisfactorily low sensitivity of screening few

discs and fast depletion of samples, multiple discs should be punched from each specimen card.

For the duration of this project the extraction protocol presented by Ahmed et al (2011) was followed, being comparable to the direct isolation of genetic material from whole blood.

1.5. Control of African Trypanosomiasis

1.6.1. Insecticide spraying of Cattle

One means of controlling trypanosomiasis is spraying of cattle with insecticide which then kills feeding tsetse flies; pyrethroids are commonly used for this purpose. Pyrethroids are compounds that are highly toxic to most insects, including tsetse flies, with only mild toxicity to mammals (FAO, 2011). This makes them suitable for widespread agricultural use and, particularly relevant to tsetse control, spraying of cattle. Pyrethrins are naturally occurring products of certain species within the genera *Chrysanthemum* and *Tanacetum* that have long been used as insecticides. There is evidence of the use of ground *C. cinerafolis* in first century AD China (Davies et al, 2007). Pyrethroids are synthetic analogues of pyrethrins that are more photostable than their natural counterpart, overcoming a major limitation to their use as insecticide.

Pyrethroids prevent closure of cell membrane Na^+ ion channels of PNS and CNS neurons, causing hyperexcitability and therefore producing spontaneous, repetitive discharges. This produces a state of sublethal excitatory paralysis (known as 'knockdown' effect) and subsequent death. Type II pyrethroids (e.g. deltamethrin) bind irreversibly to Na^+ ion channels, resulting in better kill than type I compounds (e.g. permethrin) that dissociate from the target.

Bardosh et al (2013) found that not all of the insecticide products used by a sample of farmers were effective for controlling tsetse. A number of farmers were using products containing compounds other than pyrethroids (Table 2).

	Brand	*Compound*
Acaricide products effective against tsetse flies and ticks	Alfapor© Spray and Dip	Alpha-cypermethrin
	Sypertix©	Alpha-cypermethrin
	Decatix©	Deltamethrin
	Bayticol™ Dip and Spray	Flumethrin
Acaricide products effective against only ticks	Amitix©	Amitraz
	Milbitraz©	Amitraz

	Norotraz©	Amitraz

Table 2. Insecticide products used for spraying of cattle. Adapted from Bardosh et al (2013)

Amitraz is a formamidine acaricide, and amitraz pour-on and spray products were found to be widely used for the control of ticks on cattle. The mode of action of formamidine compounds is through interaction with CNS octopamine receptors and inhibition of monoamine oxidases. (Peter et al 2006) Amitraz products are ineffective in control of tsetse flies (Hall and Fischer, 1984)

Spraying insecticide on only the underbelly and legs of cattle, which are important landing sites for tsetse (Torr et al, 2007), is known as the restricted application protocol (RAP). This dramatically decreases the amount of insecticide used, making RAP a very-cost effective means of tsetse control.

1.6.2. Trypanocidal Drug Administration

Another means of controlling trypanosomiasis is the administration of trypanocides. There are a three compounds used in the chemotherapy of AAT in cattle; each of which has therapeutic effect, prophylactic effect or both. Diminazene aceturate has only therapeutic properties. Homidium chloride has limited prophylactic effect but is administered mainly for therapeutic use. Isometamidium chloride is used for prophylaxis; it provides effective protection for up to six months (Holmes et al, 2004).

1.7. Uganda

Uganda is a nation in East Africa located between -1.5°N and 4.2°N; and between 29.5°E and 35.0°E and has a total area of 241,038 km^2, of which 43,938km^2 (20%) is water (CIA, 2016). It is bordered by South Sudan to the north, Kenya to the east, Democratic Republic of the Congo to the west and Rwanda, Tanzania and Lake Victoria to the south (Fig. 3; CIA, 2016)

The Nile River leaves Lake Victoria and flows north into Lake Kyoga in the centre of Uganda. It then flows north and then west before feeding into Lake Albert. The Nile flows out of Lake Albert and once again flows north to the South Sudanese border.

The Köppen-Geiger classification of Uganda is tropical savannah climate (Peel et al, 2007); with dry seasons running from December-January and June-August. The terrain is mostly plateau at around 1000m elevation with some mountainous regions. Lake Albert and the valley of the Albert Nile in the north-east are lower with an extreme of 621m.

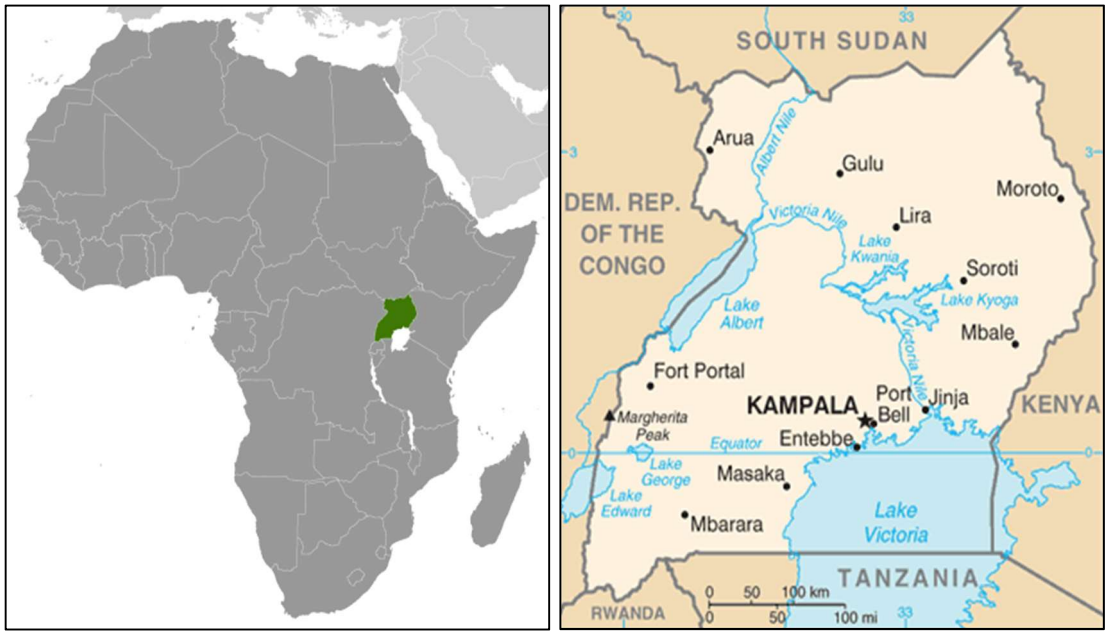

Figure 3. Map of Uganda and its location within Africa.

The population of Uganda is 37.1 million (July 2015 estimate); the urban population is 16.1%. Of the 18.58 million labour force, 82% are occupied in agriculture (CIA, 2016). Much of Uganda has fertile soils and regular rainfall. Agricultural production is high and the vegetation provides ample forage for cattle grazing.

The largest administrative division is the district, of which there are 111 in Uganda. The subdivisions thereafter are that of the county, subcounty, parish and the smallest unit is the village; an area that may have up to around 1,000 inhabitants. The lowest level of government is the Local Council (LC) 1 which is responsible a village. An LC1 Chairperson is elected for each village.

1.8. Northern Uganda

The Acholi sub-region or Acholiland is the area of Northern Uganda inhabited traditionally by the ethnolinguistic group called the Acholi. In contemporary Uganda, the Acholi sub-region is made up of Agago, Amuru, Gulu, Kitgum, Lamwo, Nwoya and Pader districts (Fig. 4). The three districts (Amuru, Lamwo and Kitgum) that make the northern half of the Acholi sub-region are included in this study.

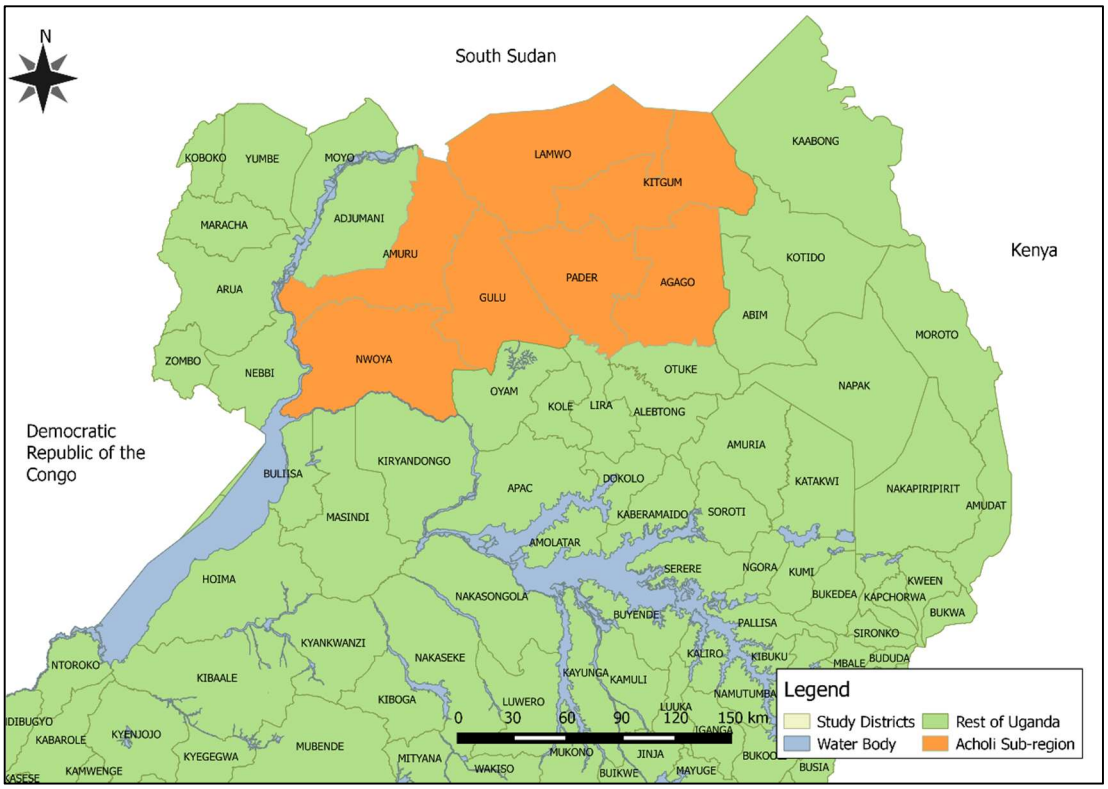

Figure 4. Map showing location and extent of the Acholi Sub-region within Uganda

The history of Acholiland and the Acholi people in post-colonial Uganda is fraught with violence. The Acholi people were victims of mass murder under the presidential regimes of Idi Amin Dada and the incumbent Yoweri Museveni, along with large scale theft of cattle (Minority Rights, 2015). In 1996 in response to the Lord's Resistance Army insurgency, which ensued from 1987 to 2006 in Northern Uganda, the Museveni government announced policy of moving the rural population of the area into internally displaced persons (IDP) camps. Ostensibly for their protection, 2 million people (over 90% of the Acholi people) forced into living in camps by 2005; around 1,000 of whom were dying per week. (Finnström, 2008).

The Acholi sub-region and the surrounding area of Northern Uganda have suffered consequences of prolonged conflict including poverty, food insecurity, neglect of infrastructure and inadequate capacity for provision of public services. The Peace, Recovery and Development Plan (PRDP) was launched by the Ugandan Government and, in conjunction with international partners and NGOs, the aim is to stabilise and improve welfare of the population of the Acholi sub-region and other areas of Northern Uganda. Two objectives of this plan are particularly relevant to this study: the rehabilitation of road infrastructure, and increasing livestock production through provision of improved breeds. (GoU, 2007)

1.9. Expanding Ugandan Human African Trypanosomiasis Foci

Uganda is unique with regards to HAT as it is the only country to be affected by both rHAT and gHAT. Cases of gHAT are reported in the north west of the country and rHAT cases have historically been confined to the south east of Uganda in Busoga region between Lake Victoria and Lake Kyoga, however events in recent years have brought about expansion of the affected area to the northwest. Fèvre et al (2001) investigated the outbreak of *T. b. rhodesiense* in Soroti district, northwest of the historic focus, which came about in December 1998, in the period following the end of the Teso Rebellion in 1994.

Civil unrest in the area in the 1980s led to migration away from the area. The land which was no longer subject to bush clearance and agricultural production reverted to its natural state, providing habitat to tsetse. With the return of stability came a migration of displaced people back to the area and GoU-funded cattle restocking was conducted as part of a series development programs to improve agricultural production. The cattle provided by these programs were in part sourced from the *T. b. rhodesiense* focus and infected cattle were introduced into tsetse infested areas (Hutchinson et al, 2003).

Fèvre et al (2001) identified the origin of cattle traded at Brookes Corner market, the main cattle market of the area, from April 1995 to December 1998. They tested whether proximity to Brookes Corner was a risk factor for sleeping sickness by collecting blood sampled from sleeping sickness case patients reporting to Serere Health Centre between December 31st 1998 and June 2nd 2000.

In total 54% of a large sample of 2796 cattle traded at Brooks Corner during this period had originated from within the south-eastern *T. b. rhodesiense* focus. At the time, there was a significant association between human cases of HAT and the distance of the patients homestead from Brookes Corner, this reach expanded with the duration of the outbreak.

The market and surrounding area are suitable tsetse fly habitat and the findings regarding proximity of human cases to Brookes Corner are in keeping with the theory that tsetse were acquiring infection near the market and transmitting the disease further afield (Fèvre et al, 2001). The results regarding distance of human cases from the market and time from the beginning of the outbreak indicate that sleeping sickness spread outwards from the market during the time period studied.

Welburn et al (2001) while validating the SRA gene as a diagnostic target for rHAT measured the prevalence of *T. b. rhodesiense* infection in cattle in Soroti during this outbreak. A small sample of 200 cattle in the district were screened for *T. b. rhodesiense* and

prevalence was estimated to be up to 18% (95% CI 12–23). This demonstrated directly through diagnosis of animal cases that the disease focus was expanding northwest.

Following the identification of the 1998 outbreak of *T. b. rhodesiense* infection in cattle in Soroti district, a control program was undertaken. Between January 2000 and December 2003 mass trypanocidal treatment of cattle in the region was carried out as well as some vector control. Fèvre et al (2005) investigated the prevalence of *T. brucei s.l.* and *T. b. rhodesiense* in cattle in Soroti district following this control intervention. Blood samples were taken from cattle in six villages in Soroti with history of sleeping sickness reporting; three villages outside the intervention area and three villages within the intervention area. They also collected samples from cattle at Brookes Corner cattle market. It was found that the prevalence of *T. b. rhodesiense* was not significantly higher in the cattle in the non-intervention area than cattle in the intervention area, suggesting that the intervention had been unsuccessful. They also found that the prevalence was significantly higher in the cattle sampled at Brookes Corner, providing more evidence in line with the findings of Fèvre et al (2001) that the spread of the pathogen through this new area was emanating from the market, introduced by infected cattle from the *T. b. rhodesiense* focus.

Fèvre et al (2005) also comment on the HAT situation in the area during the period. Since the first case reporting at Serere Health Centre in 1998 there had been 428 cases of *T. b. rhodesiense* HAT reported. It is also noted that from April 2004 onwards, there had been cases in Kaberamaido district (northwest of Soroti) and between February 2004 and January 2005 there had been 144 cases from southern Lira district (still further north). Cases in these districts show that the *T. b. rhodesiense* focus had continued to expand north.

Of the rHAT cases reporting to Serere Health Centre during this period, 67% of the 428 cases were late stage, indicating the failure of the healthcare system and of the communities to diagnose cases early. The importance of this is that clinical outcomes of sleeping sickness are better if treated in early stage and the healthcare system in the newly affected area is shown to lack the capability to promptly diagnose cases.

1.9.1. Discreet but Convergent Foci

Picozzi et al (2005) collected blood samples from 231 sleeping sickness patients living in Soroti, Kaberamaido, and Lira districts who had reported to Serere Health Centre in southeast Uganda between 2001 and 2005. Further samples were collected from 91 patients who had reported to Omugo Health Centre in Arua District (northwest Uganda) and Kiri, Ibba and Tambura health centres in South Sudan between July and September 2003. These

samples were screened using TgsGP-PCR, to detect *T. b. gambiense*, and SRA-PCR, to detect *T. b. rhodesiense.* The samples from northwest Uganda and South Sudan were, as expected, all found to be gHAT cases and the samples from southeast Uganda were found to contain rHAT cases.

These results indicated that at that time, although the two disease foci remained discreet, the areas affected by *T. b. rhodesiense* and *T. b. gambiense* had not yet converged and were 150km apart. The result of this study highlights the importance of carrying out more work to monitor the moving disease foci; to this end, von Wissman et al (2014) investigated the relationship between HAT cases in a village, and the prevalence AAT of cattle at the village level in Kaberamaido, Dokolo, Lira and Apac districts. Cattle were sampled from case villages and non-case villages across these districts and the prevalence of *T. brucei s.l.* and *T. b. rhodesiense* were measured by screening blood samples using TBR-PCR and multiplex SRA-PCR. Included in the study were: six case villages home to HAT patients reporting to either Serere hospital in Soroti or Lwala hospital, and one non-case village in a parish neighbouring each case village. In addition to these villages, the northernmost case village in Lira district was selected along with the only case village in Apac district and two non-case villages close to the district's only cattle market. Samples were collected from a total of 1428 cattle in these sixteen villages.

Of the sampled cattle, 15.5% (95%CI: 13.7–17.4%) were infected with *T. brucei s.l.* and 1.05% (95% CI 0.59–1.72%) with *T. b. rhodesiense.* Case status of a village had a significant effect on the likelihood of the village herd being infected with *T. b. rhodesiense,* with an odds ratio of 25 (95% CI = 1.2 – 520.71). These findings provide more evidence for the strong link between infected cattle and incidence of *T. b. rhodesiense* HAT and of the north-westerly expansion of the rHAT focus.

1.9.2. Consequences of Sympatric HAT Foci

The convergent foci of gHAT and rHAT in Uganda is a serious cause for concern. If the two forms of HAT were to become sympatric, not only would this mean another life threatening, economically damaging health condition for the healthcare services of the area to manage, but this would mean that HAT cases in a given location could be caused by either *T. b. gambiense* or *T. b. rhodesiense.* As the CATT is only able to detect *T. b. gambiense* and the parasitaemia in gHAT is usually too low to detect infection with microscopy, false negative diagnoses of HAT cases may occur.

As discussed in Section 1.3., it is already the case that HAT sufferers commonly require several visits to a healthcare facility before a correct diagnosis is made, and this problem may be exacerbated by the sympatry of foci. Not only does the necessity of multiple visits to healthcare facilities represent a financial cost to sufferers but also, particularly in rapidly progressing rHAT, time delays in receiving correct treatment may cause increased morbidity and mortality.

Diagnostic techniques for the two forms of HAT are different and so are the treatments of the early stage of the disease. The situation in the rHAT focus is currently that if trypanosomes are detected in the blood, a diagnosis is made and suramin administered. If the two distributions converge, this will no longer be the case; *T. b. gambiense* is not usually detected by microscopy although anecdotally parasitaemia of peripheral gHAT infections is higher in northern Uganda than reported in Western Africa. As the two human infective trypanosome species are morphologically indistinguishable (Uilenberg, 1999), cases of early stage gHAT infection may be misdiagnosed and inappropriately treated with suramin.

In order to avoid these consequences, in the event that the gHAT and rHAT foci do converge, protocols for diagnosis and treatment will require revision to adapt to the situation where HAT patients could be infected with either *T. b. rhodesiense, T. b. gambiense* or both.

1.9.3. Future expansion of the rHAT focus

Selby et al (2013) also investigated this expansion by investigating the risk of *T. b. rhodesiense* expansion to districts affected by the Lord's Resistance Army insurgency through restocking programs taking place between 2006 and 2008. Ten markets deemed to be most prominent in the northbound cattle trade were identified by DVOs. Observations at these markets were made concerning market infrastructure, sales practices and veterinary drug treatments of traded cattle. Interviews were carried out with private livestock traders, farmers, NGOs, veterinarians and market staff. Movement permit records were sought from markets for periods between mid-2006 and mid-2008. Blood specimens were collected from cattle at the ten markets between May and July 2008 and screened for prevalence of *T. b. rhodesiense.*

Movement permit records were not available for all markets but those that were available indicated that 39.5% of cattle had been sold and moved to *T. b. rhodesiense*-free districts and of particular concern, 12% were destined for districts with reported cases of *T. b. gambiense.*

These numbers were based only on official records from 8 markets of 47 identified within the area endemic for *T. b. rhodesiense*. Furthermore, it was found that traders may seek

movement permits from DVOs offices directly, that there was "illegal" movement of cattle without permits, and that requests for permits may not be representative of the entire number of cattle to be transported. This made it difficult to accurately estimate the numbers of cattle movement based on market records alone. From district records, 98.5% of cattle moved out of Lira were shown to have been destined for *T. b. rhodesiense*-free districts and 52% of these were moved to districts with *T. b. gambiense* cases. It was found that the number of cattle being traded northwards into these districts was increasing over time.

Animals infected with *T. b. rhodesiense* were identified in eight of the ten markets studied and the prevalence ranged from 3.8% [95% CI: 0-9.1] to 0% [95% CI: 0-2.3] in different markets. Using an estimated *T. b. rhodesiense* prevalence of 1.5% [95% CI: 0.9-2.0] and movement permit records, it was estimated that 434 *T. b. rhodesiense* infected cattle had been traded to districts free of the pathogen between mid-2006 and mid-2008. Of the districts which the cattle in this study were moved to, Amuru, Adjumani and Kitgum are included in my project.

Interviewed farmers and traders did not understand the importance of trypanocidal treatment in preventing the spread of sleeping sickness. Treatment of *T. b. rhodesiense* infection in cattle was seen as unnecessary as there is no clinical manifestation and therefore the animal was not perceived to be suffering from an infection. DVOs were sceptical that trypanocidal treatment of traded cattle takes place in markets and they emphasised the large amount of trade taking place outside of the market setting (Selby et al, 2013). With the numbers of infected cattle estimated to be traded, and the numbers being transported into currently *T. b. rhodesiense*-free areas, this information that trypanocidal treatment is not given to these animals before transport paints a worrying picture of the possibility of future expansion of *T. b. rhodesiense* into *T. b. gambiense*-affected districts.

1.10. Risk Factors for Infection in Animals

Cattle introduced to northern Uganda may be a factor in an evolving epidemiology of AAT and a possible arrival of *T. b. rhodesiense* in the area.

Varying susceptibility to trypanosome between different breeds has previously been studied. Magona et al (2004) investigated the prevalence of trypanosome infection in Nkedi Zebu and Ankole in a sample of cattle in Soroti district. They also studied how prevalence varied across sex, age and functional status (milk, draught or breeding) groups. They found a higher prevalence of trypanosome infection in the Ankole cattle (10.8%) than in the Zebu (7.9%) though the difference was not significant [$X^2 = 2.28$; df = 1; $p = 0.130$].

Anene et al (1991) found that in a comparative study of clinical signs, haematology and prevalence of trypanosomiasis in cattle in Nigeria, that 44% (23/52) of adult Friesian cattle were infected whereas 6.9% (2/29) of Friesian calves and 15% (3/20) Zebu were infected. If combining the numbers for Friesians, the overall prevalence is 30.8% (25/81). The Fisher's Exact Test *p*-value for the comparison of prevalence in Friesian and Zebu cattle is 0.028 and the odds ratio is 4.49. Friesian cattle are among the exotic breeds that have been introduced into the study area.

Cattle introduced to northern Uganda may be a factor in an evolving epidemiology of AAT and a possible arrival of *T. b. rhodesiense* in the area. If the northern districts are being restocked with susceptible breeds as well as with infected cattle (Fèvre et al, 2001; Selby et al, 2013) then this may increase transmission of trypanosomiasis and facilitate expansion of *T. b. rhodesiense* into the north-western *T. b. gambiense* focus.

This possible increased susceptibility of AAT may be problematic due to the desirability of Ankole and exotic cattle owing to their increased productivity over the indigenous Zebu. As highlighted by Magona et al (2004), Ankole develop into larger animals, making them more valuable at market and more useful for draught power, and the reason of the introduction of European breeds is their capacity for milk production (Kabunga, 2014).

Use of different grazing systems may also impact on AAT risk. MacLennan (1979; cited in ILCA, 1979) states that zero-grazing of cattle in fly-proof shelters can protect cattle from trypanosomiasis risk compared to cattle that are extensively grazed. The potential for some grazing systems to pose increased risk of infection warrants investigation of this association.

The prospect of the herds of this region becoming increasing made up of exotic breeds warrants further investigation of the possible association between breed and trypanosusceptibility.

1.11. Aims and Objectives

The aims of this study are: to ascertain prevalence of *T. brucei* s.l. infection in the northernmost districts of Uganda as a surrogate measure of AAT prevalence and to generate a profile of the herd structure and cattle management practices in the area, and how they relate to risk of increasing AAT prevalence and introduction of *T. b. rhodesiense* to the area.

The objectives that will be met in order to reach these aims are: to screen a representative sample cattle blood specimens for the presence of *T. brucei* s.l. genetic material in order to calculate prevalence and infection data for individual animals; to examine attitudes and

knowledge of cattle farmers regarding trypanosomiasis control, in particular spraying of cattle with insecticides and administration of trypanocides; to examine cattle trading practices; and to investigate herd structure and relative risk of *T. brucei* s.l. infection in different groups of cattle.

2. Methods

This chapter will describe the collection of specimens and data in the field, the subsequent molecular analysis of the specimens undertaken to estimate the prevalence of *T. brucei* s.l. infection and the analysis of the data generated.

2.1. Study Design

The specimens described herein were collected as part of a larger, ongoing study. This study is a pilot for a study designed to establish estimates of baseline prevalence of *T. brucei* s.l. infection in Ugandan cattle against which the success of future control interventions can be measured. The sampling strategy being identical to that of the larger encompassing study apart from its restriction to a smaller number of districts.

The study area focuses on the northern-most districts of the Northern Region of Uganda, many of which border South Sudan (Fig. 5).

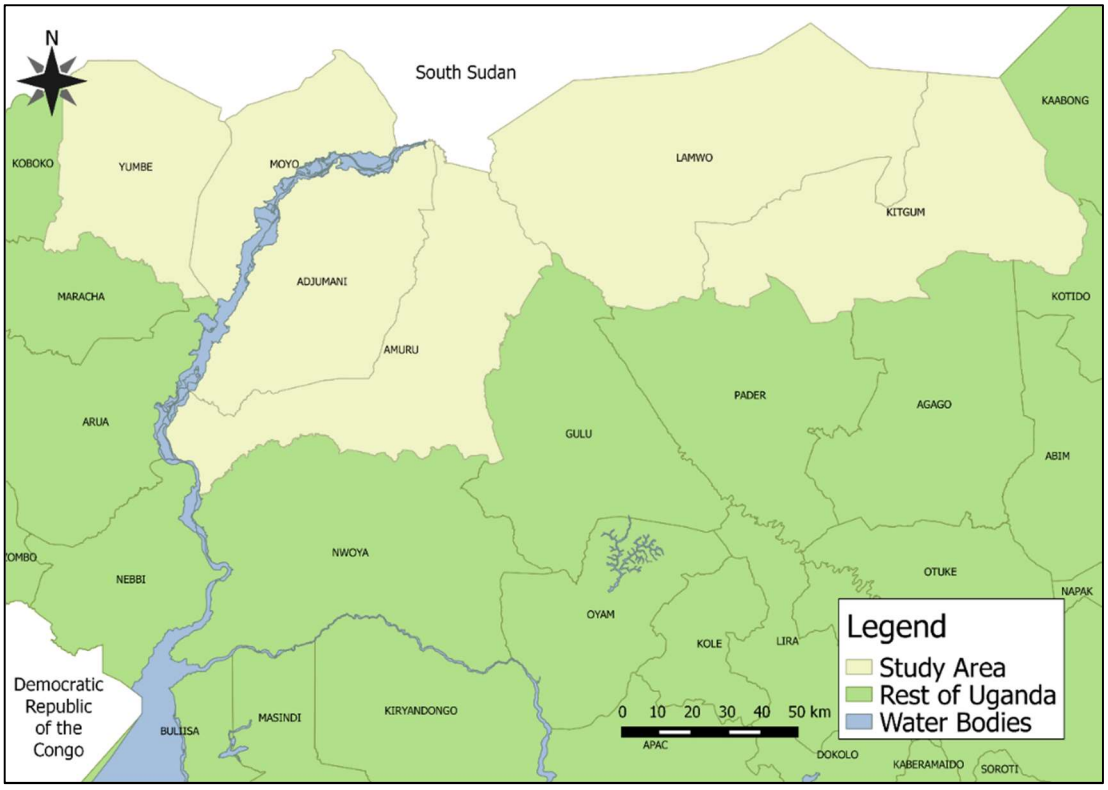

Figure 5. Districts included in this study: Adjumani, Amuru, Kitgum, Lamwo, Moyo and Yumbe

These districts have been heavy restocked with livestock in recent years, often with cattle from areas of Uganda where *T. brucei* s.l. infection prevalence is high and where *T. b. rhodesiense*-infected animals are present (Selby 2013). Although legislation is in force requiring trypanocidal treatment to be administered to animals prior to transport from these high risk districts, there is an associated risk with these restocking activities. As the

Ugandan *T. b. rhodesiense* focus has expanded north within recent years (Fèvre et al, 2001; Picozzi et al, 2005; Selby et al, 2013), monitoring for infected cattle in these northern-most, high-risk districts is of increasing importance in order to revise the reach of this infection.

Two stage cluster sampling was used to generate the sample for this study; the village (administrative unit) was used as the primary sampling unit for the study and individual animals owned by residents of each selected sample village were the secondary units. The village as an administrative unit is a geographical area which is governed by an LC1 Chairperson, this being the lowest level of local government in the Ugandan political system. The village was used as the primary sampling unit for a number of reasons. It is a rigid, well defined geographical area of which individuals are resident to only one. By extension of this, cattle are therefore only owned by residents of one village, avoiding ambiguity with regards to the location of data from individual animals and inclusion of animals in the study. For village level data, the village as an administrative unit and the Local Council 1 (LC1) chairperson as the representative of this unit provides a central and reliable source of data for the village as a whole and for individual farmers residing within.

The primary units (villages) were selected at random from the entire sampling frame of villages within the northern districts to be studied. All animals belonging to residents of the selected villages were included in order to avoid bias in the cattle presented and to provide complete data for the local herd. As well as blood specimens, information on the characteristics of the animals presented were also collected (see Section 2.2.2.).

The secondary units were selected systematically, with every fourth specimen from each village selected for PCR analysis to generate infection data based on 25% of each village herd (see section 2.3.4.). A minimum of fifty specimens from each village were screened, so in villages where the number of specimens collected was less than two hundred, more than 25% of animals were screened. For villages where less than fifty specimens were collected, every specimen from the village herd was screened. The reason for aiming to screen at least fifty specimens for each village was to lend more power to statistical analysis of this data and to narrow confidence intervals for estimates of prevalence. Ideally, every specimen collected would be screened but screening a sample of these specimens was the best trade-off between statistical power and expenditure of time and resources.

2.2. Field Work

Field work in Northern Uganda was carried out between May and June 2015. The team that conducted the field work consisted of a University of Edinburgh Research Associate, a

University of Edinburgh MSc(R) Student, two Ugandan veterinarians and a driver. The tasks of the field work were: mobilisation of farmers and cattle for specimen collection; and collection of blood specimens and cattle data. For each village in the sampling frame, one day was allocated for specimen collection.

2.2.1. Mobilisation

Upon arrival in each of the districts within which were study villages, the team met with the District Veterinary Officer (DVO), chief administrative officer (CAO) and resident district commissioner (RDC) and made them familiar with the work that was to be carried out in their district. Following these meetings, the field team, accompanied by a member of the DVO's staff, would travel to each subcounty containing villages in the sampling frame and liaised with the Subcounty Chief to familiarised with the project and gain any relevant information regarding the study villages within their subcounty. From here, the field team travelled to each of the study villages and met the LC1 Chairperson. The team met with LC1 officials in order to secure their full engagement, approval and awareness of the project before specimen and data went ahead.

During this meeting, the purpose and aims of the work to be conducted in village were discussed and a date was arranged on which the field team would return to the village to collect blood specimens from every cow owned by residents of that village. This process was necessary as it provided the LC1 Chairperson time to mobilise all of the cattle-owning residents of their village to bring all of their cattle to a central location on the appointed date. If residents of the village were present when the field team met the LC1 chairperson, as was often the case, the project was presented to them in order to ensure awareness and engagement in the project as much as possible while addressing any concerns or queries (Fig. 6). As an incentive to attend the specimen and information collection with their animals, cattle owners were offered a de-worming treatment (an albendazole drench) free of charge for each animal presented; regardless as to whether a blood specimen was or was not collected.

Figure 6. Familiarisation of the LC1 Chairperson and cattle owners with the project

As well as mobilising the cattle owners in the village, the LC1 Chairperson was given a 'village questionnaire' to complete. These questionnaires provided information regarding veterinary drug usage, cattle trade information and an overall cattle census in the village

In the intervening period between the meeting and day of specimen collection, the LC1 Chairperson was contacted via telephone to resolve any queries, receive updates on the progress of the mobilisation of cattle owners, and acquire estimates of the number of cattle to be sampled in order to carry an adequate amount of equipment to the village on the day of specimen collection.

The DVO's assistant was crucial in achieving successful mobilisation of cattle owners. In many areas, the local population did not speak any languages in which the field team were able to communicate. In these circumstances, the DVO's assistant facilitated communication with the LC1 Chairpersons and with village residents, as their standard of both English and the local language was universally very good. The DVO's assistant was often also familiar to some of the cattle owners from previous activities or was usually at least a local to the district and this made the LC1 Chairpersons and cattle owners more cooperative.

2.2.2. Specimen collection

On the pre-arranged date, the LC1 Chairperson mobilised the cattle owners of their village and arranged that they assemble at one location in the village. The reality in many villages was often that while many owners did coalesce at a single location, a number did not and it was often necessary to travel to other herds located elsewhere in the village or to local grazing sites in order to collect specimens from the entirety of the village herd. GPS coordinates were recorded at a central location for each of the village sites to be used for the generation of maps to visualise the distribution of villages (see Section 2.4.1.).

Animals were restrained by hand and by using ropes; the cooperation of the cattle owners was often crucial in achieving this. In some villages, a cattle crush (stall in which animals could be held) was constructed which aided in the restraining of the cattle. Unilet® ComforTouch Lancets (Owen Mumford) were used to pierce the marginal ear vein of each animal (Fig. 7), blood was collected using heparinised capillary tubes and was applied to Whatman® FTA® cards (Fig. 8).

Figure 7. A cow is restrained and a blood specimen is collected in a heparinised capillary tube
Figure 8. (Right) FTA® Card with four blood specimens applied

Alongside each blood specimen applied to the FTA® cards, data regarding various characteristics of the animal were recorded. Table 3 outlines the characteristics recorded and the categories to which animals belonged.

Characteristic	Category
Sex	Male; Female
Age	Calf; Yearling; Mature
Breed	Zebu; Ankole; Exotic; Mixed
Body condition	Lean; Medium; Fat
Used for Traction	Yes; No
Grazing system	Zero grazing; Tethered grazing; Communal grazing

Table 3. Information collected regarding sampled animals

FTA cards with applied blood were air-dried for a period of 24 hours and packaged in Whatman® FTA® Multibarrier Pouches along with MiniPax® absorbent packets (Sigma) in order to keep the pouch contents dry and avoid deterioration of blood specimens. Pouches were labelled with information regarding the village and the date of specimen collection, and along with completed 'village questionnaires', were mailed to the University of Edinburgh (UoE). The conditions of the import license obtained from the Scottish Government stipulated that each batch of FTA cards sent from the field to UoE for analysis must be marked with pre-determined information.

2.3. Laboratory Screening

The laboratory screening of the blood samples collected in the field followed the process shown by Fig. 9.

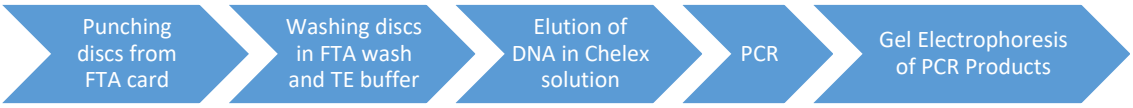

Figure 9. Process of laboratory screening of blood specimen

2.3.1. Punching Discs from FTA Card

A section of lab bench was cleaned with 70% ethanol and bleach then covered with cling film to create a clean work area. Punches and tube racks containing 1.5ml microcentrifuge tubes were sterilised in a UV Sterilising PCR Cabinet (UVP®) for a period of no less than 30 minutes. Whatman® FTA Cards were removed from the Whatman® FTA® Multibarrier Pouch in which they had been transported and five 3mm discs were punched from the area of the Whatman® FTA card to which blood had been applied (see fig. 8). The discs punched from each specimen were deposited into one of the 1.5ml microcentrifuge tubes. A negative control was collected at this stage of the screening by punching five discs from blank filter paper. These blank punches subsequently underwent the same process as the blood specimen punches.

In order avoid cross-contamination between specimens, the punch was cleaned by punching discs from blank filter paper between specimens. Additionally, the punches were cleaned with 70% ethanol and sterilized in a UV Sterilising PCR Cabinet (UVP®) after every 40 specimens punched.

2.3.2. Removal of Contaminants from FTA Card (Picozzi et al, 2002)

The discs (five punches removed FTA cards) underwent two ten minute washes in FTA Purification Reagent (Whatman®) and two ten minute washes in 1x Tris-EDTA buffer solution (prepared in 500ml batches: 5ml of 100x Tris-EDTA buffer solution in 495ml of Milli-Q® purified water). FTA Purification Reagent was used to remove PCR inhibitors and contaminations from the card discs and facilitated release of DNA from the FTA matrix. The discs were washed with 10x Tris-EDTA buffer solution to remove FTA Purification Reagent (a potent PCR inhibitor) from the discs.

The washed specimens were then transferred from each microcentrifuge tube into 0.2ml 8-strip PCR tubes which had been sterilized in the UV sterilizing cabinet. The discs were heated in an incubator for 45 minutes at 37°C or left overnight to dry.

2.3.3. Elution (Becker et al, 2004)

To each PCR tube containing the punched discs, 100µl of 5% (w/v) aqueous suspension of Chelex® 100 was added using a large-bore pipette tip. The tubes were then heated at 90°C for 30 minutes to elute the DNA from the discs.

In order to preserve DNA structure, eluates were stored at -20°C until PCR analysis if this was not to be done immediately after elution.

2.3.4. Polymerase Chain Reaction

Specimens were screened with TBR-PCR for *T. brucei* s.l. (Moser et al, 1989) to detect infection of the animal with *T. brucei* s.l. The components of the mastermix for this PCR are shown in Table 4.

Reagent	***Volume for screening 1 sample (µl)***
Water	17.205
Primer stock (10µM of each primer)	1
dNTPs, 25mM each	0.15
5x MangoTaq™ Coloured Reaction Buffer	5
$MgCl_2$, 50mM solution	0.45
MangoTaq™ DNA Polymerase	0.2

Table 4. Components of TBR-PCR mastermix

The size of the PCR product of TBR-PCR is 177bp; the sequences of the primers used for TBR-PCR are shown in Table 5.

Primer Name	***Sequence***
TBR1	5' – GAA TAT TAA ACA ATG CGC AG – 3'
TBR2	5' – CCA TTT ATT AGC TTT GTT GC – 3'

Table 5. Sequences of TBR-PCR primers

PCR mastermix without Taq polymerase was produced in batches for 55 samples in 1.5ml microcentrifuge tubes and kept on ice. The 25µl PCR reaction mixture consisted of 24µl mastermix and 1µl DNA eluate for each specimen screened.

The wash control (1µl of eluate from washed and eluted blank discs), a further negative control of 1µl of water (to detect contamination of mastermix with *T. brucei* s.l. DNA) and a positive control of 1µl of *LIRI 039 T. b. rhodesiense* genomic DNA were included with each batch of specimens screened.

TBR-PCR was conducted with an initial denaturing step of 3 minutes at 94 °C, followed by 35 cycles of: 1 minute at 94°C (denaturation), 1 minute at 55°C (annealing), and 30 seconds at 72 °C (extension.) There was a final extension step of 5 minutes at 72°C.

PCR products were refrigerated at 4°C prior to gel electrophoresis analysis in order to prevent breakdown of the DNA structure of products.

3.4.5. Agarose Gel Electrophoresis

Gel electrophoresis was used for analysis of PCR products. Each gel was using 100ml of 1.5% (v/w) agarose (Bioline) in 1x Tris-Borate-EDTA buffer solution. The 1x TBE was produced in batches of 2 litres; 200ml of 10x Tris-Borate-EDTA buffer solution (Sigma) in 1800ml water. The 1.5% agarose in TBE was heated for 2 minutes until dissolved. To stain DNA in order that any bands of DNA could be visualised, 4µl of GelRed™ (Biotium) was added to each 100ml of molten agarose gel. The molten gel was allowed to cool for 5 minutes and then poured into gel trays with 30-lane combs and left to set for 20 minutes.

Into each lane of the gel, 12µl of PCR product was pipetted, and 6µl of ladder (Fisher BioReagents™ exACTGene™ Low Range plus DNA Ladder) was pipetted into the wells at each end. Both positive controls and the negative control (12µl of each) were pipetted into the lanes to the right of the ladder. Negative controls and the positive control were separated by an empty lane to avoid spillover between lanes. Fig. 10 shows the layout of lanes on a gel. Lane 14 and lane 16 have 177bp bands, indicating that the specimens in those lanes contain *T. brucei* s.l. genetic material.

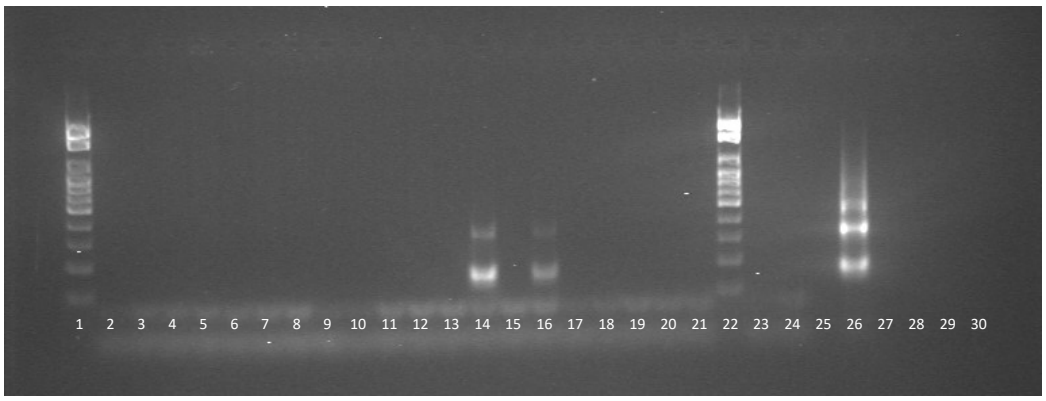

Figure 10. Position of different lanes in agarose gel. 1 = ladder, 2 to 21 = specimens, 22 = ladder, 23 to 24 = negative controls, 26 = positive control, 27 to 30 = unoccupied

Gels were placed in the electrophoresis cell, submerged in 1x Tris-Borate-EDTA buffer solution and connected to a power supply. A 110v current was passed through the gel for 50 minutes. Gels were then imaged in a Gel Doc 2000 transilluminator using Quantity One™ (Bio Rad) software to capture images of the gels. The position of any bands present in the specimen lanes were compared to the bands in the ladder lane allowing the determination of the length of the PCR product producing the band.

2.4. Geographic Information System Maps

The location of each of the study villages was recorded using GPS at a central point in the village. These coordinates were used in combination with village prevalence data and information from questionnaires regarding trading practices and use of insecticide products for spraying cattle to produce maps. The open-source software QGIS was used to produce these maps and images to represent this information.

2.5. Statistical Analysis

Microsoft Excel (version 15.0) was used for data entry, storage and management. This software was also used to produce charts for visualisation of this data. Data was exported to IBM® SPSS® Statistics (version 22) for descriptive analysis and generation of cross-tabulations and frequencies. WinPepi (version 11.62) was used for a number of statistical tests using the frequencies generated by SPSS, viz. calculation of Spearman's rho; Pearson's Correlation Coefficient; Pearson's chi-square; Fisher's exact test; calculation of infection prevalence and confidence intervals; and odds ratios.

3. Results and Commentary

The results of the analysis of village level questionnaire responses is the first section of this chapter. Analysis of individual animal data follow. Findings from the laboratory screening of blood specimens are described in the third section and the final section is the risk factor analysis that involves both individual animal data and *T. brucei* s.l. screening data. Commentary on the findings is also found accompanying each of these outlined sections.

3.1. Questionnaire data

Of the thirty-nine study villages, thirty-six returned questionnaires. The questionnaires included information regarding spraying of cattle with insecticide products and administration of trypanocidal drugs to cattle by farmers in the village. Information about cattle trading practices was also gathered.

3.5. Insecticide Spraying

3.5.1. Question: 'Are farmers spraying their cattle?'-

Of the village level questionnaires returned, (n=36) twenty-one (58.33%) reported farmers in the village spraying cattle with insecticide products. The products identified as being used by farmers are described in Table 2.Every village that reported farmers spraying their cattle with insecticide reported the exclusive use of manual methods of spraying. Hand sprayers, foot pump sprayers and/or knapsack sprayers were the methods identified.

Only five villages (13.89%) reported using pyrethroids products effective for controlling tsetse flies. The products used in each of these villages are listed in Table 6. Fig. 11 shows the location of villages using these products. The remaining thirty one villages (86.11%) were not spraying cattle with insecticide effective for tsetse fly control.

In sixteen villages (44.44%%) it was reported that cattle owners spray their cattle with products that are effective against ticks but ineffective against tsetse flies of which fourteen villages (38.89%) reported famers exclusively using products ineffective for control of tsetse flies. These products were Amitix©, Milbitraz© and Norotraz©; they are all amitraz products. The use of Amitix© was widespread across all districts in the study. Thirteen of the sixteen villages using amitraz products were using Amitix©. Two villages in Adjumani district, Pawinyo and Mirieyi-1 were spraying with Milbitraz©.

Elsewhere, in one village (2.78%), Mazaangwa (Adjumani district), the product that was reportedly used by cattle owners for spraying their cattle was "Albafast." This is likely referring to Albafas, an oral suspension of the anthelminthic compound albendazole. This

would not be used for spraying cattle and this response was likely due to a misinterpretation of the question.

In the other village, Beyomabor (Lamwo district), the only insecticide product identified as being used for spraying cattle was unrecognised. As well as using Amitix©, cattle owners were reported be using a product named as "Renacade". Following an extensive search, no information on this product was found and therefore it could not be identified as being either effective or ineffective for tsetse control.

Village (District)	Products effective for tsetse control	Products ineffective for tsetse control	Products not used for spraying*/unidentified products†
Pawinyo (Adjumani)		Milbitraz©	
Mazaangwa (Adjumani)			Albafas*
Mirieyi-1 (Adjumani)		Milbitraz©	
Asisi (Adjumani)		Amitix©	
Teddi (Amuru)	Decatix©		
Palukere East (Amuru)		Amitix©	
Pakuma (Amuru)		Norotraz©	
Ladwogi (Kitgum)	Sypertix©	Amitix©	
Munuotam (Kitgum)	Sypertix©		
Bardyang (Kitgum)		Amitix©	
Pawich (Lamwo)		Amitix©	
Beyomabor (Lamwo)		Amitix©	
Pobudi (Lamwo)		Amitix©	
Kamama (Lamwo)		Amitix©	
Larach Odong (Lamwo)		Amitix©	
Paubu (Moyo)	Alfapor©; Bayticol™		
Matu (Yumbe)		Amitix©	
Entebbe (Yumbe)		Amitix©; Norotraz©	
Kiri (Yumbe)		Amitix©	
Awijia (Yumbe)		Amitix©; Norotraz©	
Wabanga (Yumbe)	Alfapor©	Amitix©	

Table 6. The insecticide products used for cattle spraying in study villages

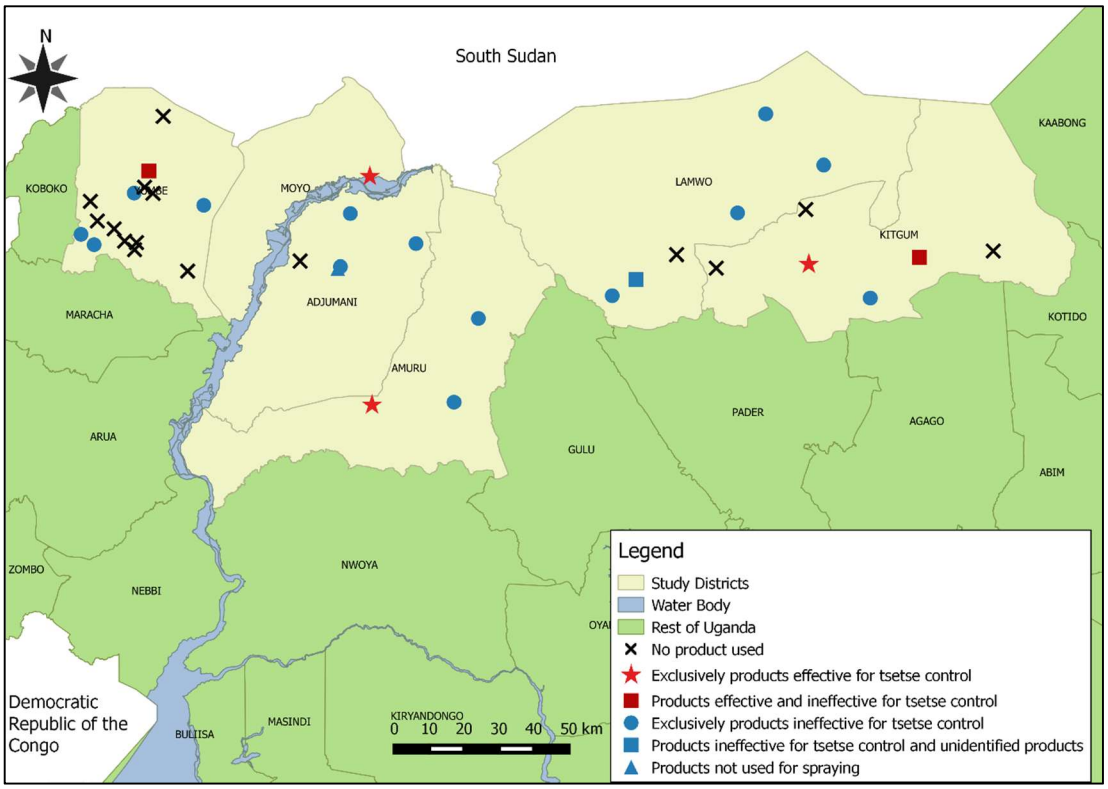

Figure 11. Locations of villages using Insecticide products for cattle spraying

3.5.2. Question: 'What are the major drivers of spraying?'

As well as information about the insecticides used for spraying, the questionnaire included information regarding attitudes to insecticide spraying. Responses to this first question can be grouped into the following categories, categories are ranked in order of frequency of response (n) from villages:

- Reference to control of ticks/tick borne disease (15)
- General animal health/productivity/ability to provide draught power (5)
- Specific reference to control of tsetse fly/nagana (2)
- Reference to nonspecific terms e.g. 'insects', 'flies' (1)

A number of villages gave responses that fall into multiple categories or gave no response. Some of the respondents (n=5) had given inappropriate responses due to misinterpreting the term "driver" as method/equipment and gave responses such as hand sprayer.

Only two of the villages (Asisi in Adjumani district; Wabanga in Yumbe district) reported that spraying of their cattle with acaricides was driven by the presence of tsetse flies/nagana. This suggests a lack of understanding or misinformation among farmers of the action regarding the products being used.

3.5.3. Question: 'What might encourage farmers to spray more?'

Responses to this second question can be grouped into the following categories, categories are ranked in order of frequency of response (n) from villages:

- Maintenance of general animal health, prevention of unspecified disease and promotion cattle productivity (10)
- Increased accessibility to effective, affordable acaricides and of spray equipment (4)
- Reference to control of ticks/tick borne disease – Chokokobidi, Mazaangwa, Kiri, Paubu (4)
- More extension worker contact (1)
- Reference to control of insects (1)
- Reference to control of tsetse fly/nagana (1)

A number of villages gave responses that fall into multiple categories and some villages gave no response.

Only one village (Wabanga in Yumbe district) reported that spraying of cattle with acaricides would be encouraged by an outbreak of nagana.

The responses to these two questions highlights a lack of awareness of the transmission of AAT and the importance of controlling the tsetse vector. The products used by farmers, the widespread use of Amitix© in particular, and the motivations for driving use of these products demonstrate the importance of tick control and prevention of tick-borne diseases for these farmers. In general, this clearly takes precedence over AAT control in the minds of farmers in this area.

3.6. Trypanocide Use

3.6.1. Question: 'Are farmers treating their animals against nagana?'

As well as information regarding tsetse control, the survey included questions regarding use of trypanocidal drugs for the treatment of AAT in cattle.

Of the returned questionnaires (n=36), 3 villages (8%) reported that the cattle owners administer drugs to their cattle for treatment of AAT. The villages that reported cattle owners in the village treating their animals for AAT were: Wabanga (Yumbe district), Kiri (Yumbe district) and Asisi (Adjumani district).

3.6.2. Question: 'If farmers are treating their animals against nagana, what products are used?'

In the three villages in which farmers administer drugs for treatment of AAT diminazene is the compound used. Diminazene is a trypanocide only suitable for therapeutic use. The compound is rapidly excreted, therefore having very limited prophylactic activity. Diminazene's trypanocidal action has yet to be fully elucidated but it has been found that the compound binds to kDNA, causing ultrastructural modifications to the kinetoplast. (Brack et al, 1972) Despite no reference to tsetse or trypanosomiasis control as drivers of insecticide spraying, farmers in Kiri village reportedly treat their cattle with diminazene aceturate. The products reportedly used by farmers in these villages are shown in Table 7.

Village	*Trypanocidal Drugs used*	*Non-trypanocidal drugs used*	*Unidentified*
Wabanga	Diminakel® (Diminazene diaceturate)	Oxytetracycline	'Norbok' 'Anamycin'
Asisi	Diminazene aceturate	Oxytetracycline	
Kiri	Diminazene diaceturate		

Table 7. Drugs used by farmers in different villages to treat nagana

Two of the three villages that reported farmers administering drugs for the treatment of AAT also reported use of drugs ineffective in treating AAT. Farmers in Wabanga reportedly administer oxytetracycline, 'norbok' and anamycin for treatment of AAT. Farmers in Asisi also administer Oxytetracycline. This is in keeping with their use of both Alfapor and Amitix.

Oxytetracycline is a broad spectrum tetracycline analogue isolated from the actinomycete *Streptomyces rimosus* (NCBI, 2016a) Oxytetracycline is not recommended for treatment of trypanosomiasis, however, there is evidence that the compound does possess a degree trypanocidal activity, probably due to ribonucleotide reductase inhibition. (Johnson and Ekanem, 2002; Ekanem and Adeniran, 2003) The administration of oxytetracycline by farmers in these villages is in keeping with the use of Amitix© to spray their cattle (see Section 3.5.1.). Amitix© is effective in the control of ticks (see Table 2) and oxytetracycline is effective in the treatment of the tick-borne disease bovine anaplasmosis (Ristic, 2012).

'Norbok' may be a reference to Norbrook Ltd, a veterinary pharmaceutical company. A number of products are produced by Norbrook Ltd including Norotryp®, a diminazene aceturate product. (Karanja et al, 2002) If, in fact, this is a reference to Norbrook Ltd, it

cannot be assumed that Norotryp® is the referenced drug particularly when Norbrook Ltd. also produce an oxytetracycline formulation. (Norbrook, 2016)

'Anamycin' may be a reference to kanamycin, an aminoglycoside antibiotic isolated from *Streptomyces kanamyceticus*. The mechanism of aminoglycoside action is through binding to the bacterial 30S ribosome subunit, causing inaccurate translation of rNA and therefore inhibiting protein synthesis (NCBI, 2016n). Maina et al (1998) found that kanamycin exhibits trypanocidal activity *in vitro* but there is a lack of research into the efficacy of this drug for AAT treatment. The drug is not recommended for use and it would be interesting to know the reasons behind the use of this drug by cattle farmers.

The use of these compounds for the ostensible treatment of nagana further demonstrates a deficit in the understanding of aetiology and treatment of AAT highlighted by the products used in spraying for tsetse control. The products used also demonstrate the importance of preventing tick-borne diseases.

3.7. Cattle Trade

Most villages reported farmers purchasing cattle at local markets within the district. Others reported farmers trading cattle locally; either door to door or at cattle craals. Some reported that cattle traded this way were transported to different districts. This trading occurs outside of the market system. The relevance of this is that a Ministry of Agriculture directive states that cattle purchased must be treated with trypanocidal drugs at the point of sale before veterinary officials will issue movement permits to cattle traders (Wendo, 2002). If cattle are traded outside of the market system, there is no way of enforcing this directive. Within the remit of this research, no information regarding volume of trade, only trading practises, was collected using the questionnaire and as such, the scale of the trading network cannot be estimated.

A number of villages reported farmers purchasing cattle from distant districts, not the district in which they reside or an adjacent district. Seven villages in Lamwo and Kitgum districts reported their farmers purchasing cattle at Amach market in Lira district (Fig. 12). The distances from these villages to Amach market ranged from ≈125km to ≈180km. Two further villages, Omuna and Pawinyo, reported farmers purchasing cattle from unspecified locations in Lira district (Fig. 14).

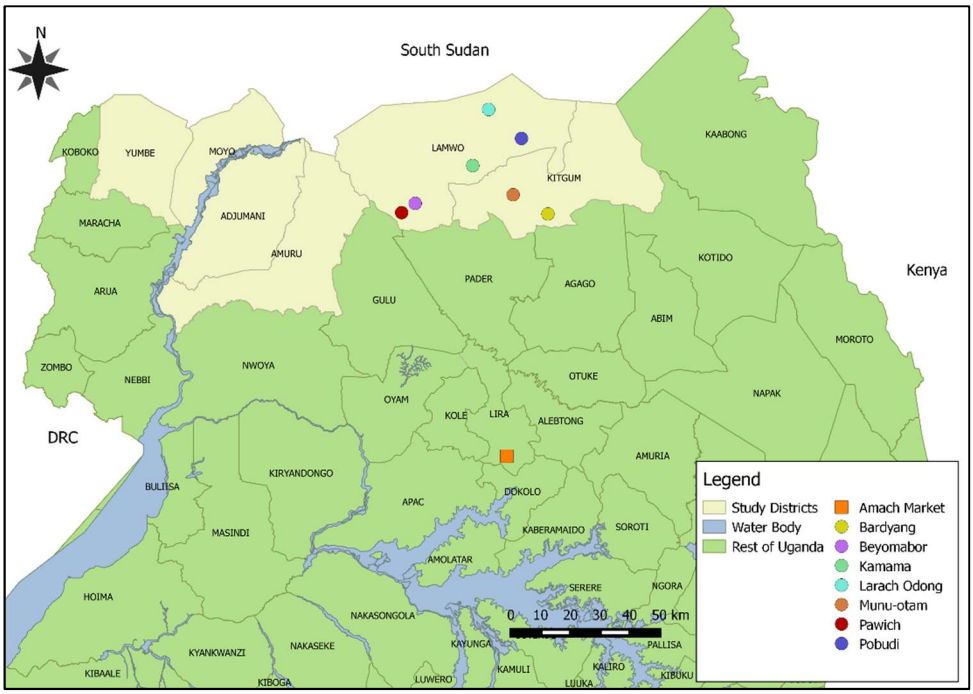

Figure 12. The location of Amach market and villages reporting that farmers purchase cattle at this market.

As well as purchasing cattle from Amach market, farmers in Pawich (Lamwo district) reportedly purchase cattle from Arapai market in Soroti district. This is ≈200km away from the village. (Fig. 13)

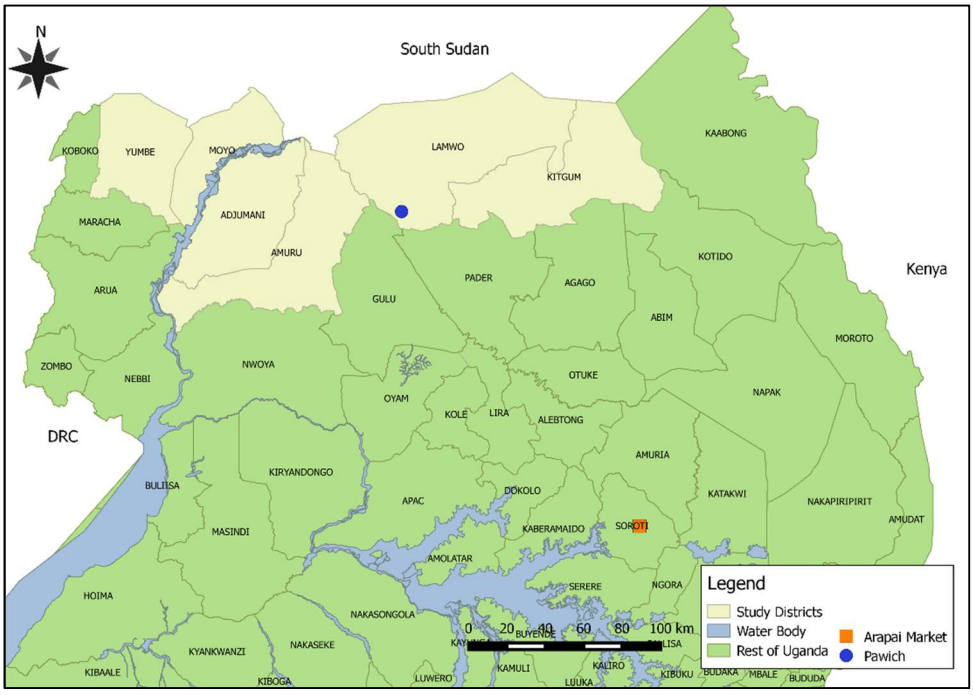

Figure 13. The location of Arapai market and Pawich village.

Omuna (Kitgum district) and Pawinyo (Adjumani district) reported that farmers purchase cattle from Lira district but did not disclose if this is from cattle markets or individuals outside of the market system.

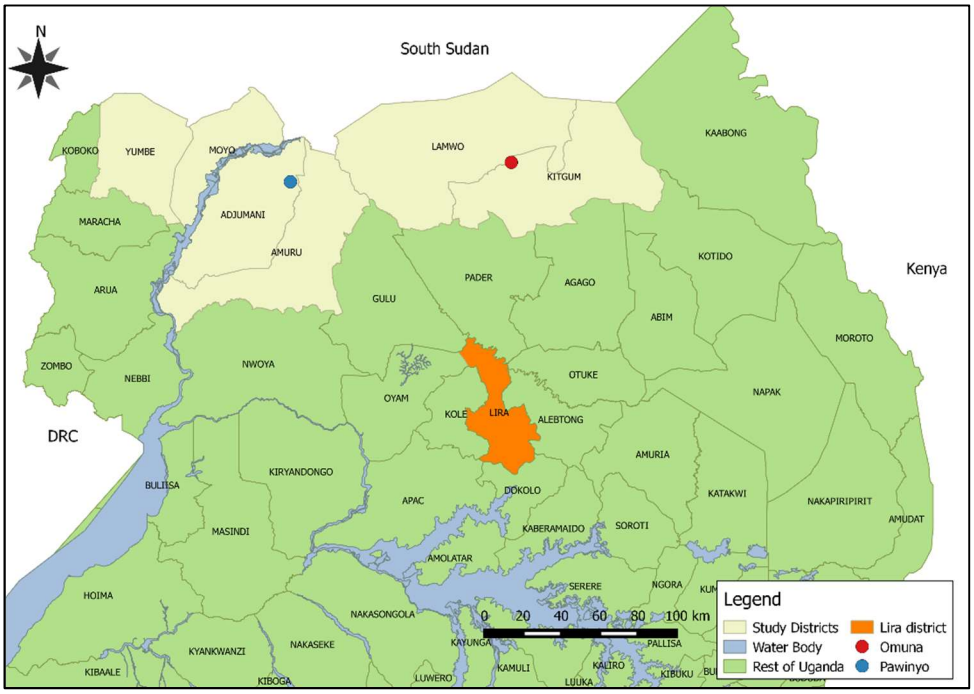

Figure 14. The location of Lira district and villages reporting that farmers purchase cattle from unspecified locations within this district

The significance of farmers in the study villages purchasing cattle in Lira and Soroti is that these districts have been found to be host to *T. b. rhodesiense* infected cattle. Selby et al (2013) screened specimens from 2006-2008, collected at cattle markets in newly *T. b. rhodesiense* affected districts. At that time, prevalence of *T. brucei* s.l. was found to be 0.6% in cattle present at Arapai market and 6.5% in cattle present at Amach market. Prevalence of *T. b. rhodesiense* was 1% in cattle present at Amach market. Farmers purchasing cattle at these markets may be at risk of introducing *T. b. rhodesiense* infected cattle to their local areas in the absence of treatment.

One village (Katum, Lamwo district) reported that farmers purchase cattle from Mach Odwogo in Oyam district. The exact location of Mach Odwogo was not known, but the closest and farthest points in Oyam district are ≈80km to ≈145km away from Katum (Fig. 15).

Oyam district has been identified as being at risk of introduction of *T. b. rhodesiense* by movement of infected cattle. The district has not historically been affected by rHAT but is adjacent to Kole and Lira districts where rHAT cases have been reported. (Bardosh et al,

2013) Again, as with other markets and districts, the scale of trade cannot be estimated from the currently available data, but if movement of cattle from this district were to continue, a time may come when cattle in Oyam are infected with *T. b. rhodesiense* and these cattle are then there is a risk of the introduction of these cattle into currently unaffected districts.

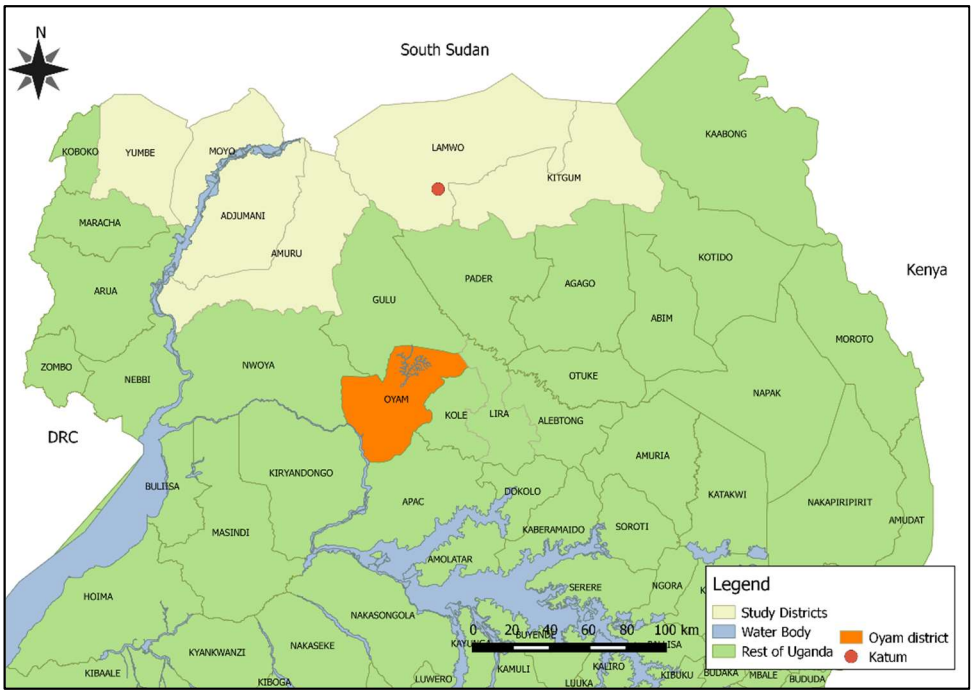

Figure 15. The location of Oyam district villages reporting that farmers purchase cattle from Mach Odwogo within this district.

3.2. Individual Animal Data

3.2.1. Herd Size

With regards to the size of the villages herds in the study, there were two measures available: the number of cattle from which specimens were collected (henceforth 'Cattle Sampled'; and the number of cattle that the LC1 chairperson reported to be owned by farmers in the village (henceforth 'LC1 Estimate'). No herd size estimate was provided by the LC1 chairperson of Makere village in Lamwo so this village was excluded from analysis of herd size.

The total number of cattle sampled was 7406 from 39 villages. When excluding Makere village from analysis of village herd size, the total number of cattle sampled is 7326 and the number of villages is 38. The average of all village 'Cattle Sampled' values (n=38) was 192.79 (SD = 103.53) and the average 'LC1 Estimate' (n=38) was 276.61 (SD = 194.15). The average 'Cattle Sampled' was 0.7 times the size of the average 'LC1 Estimate'.

A number of villages were considered to be outliers with regards to discrepancy between the LC1 estimate and the cattle samples. The 'cattle sampled' estimates may be lower than the

actual village herd size due to the logistical reality of field work that there is a limited amount of time to collect data. In this study there was one day per village to collect specimens. A number of variables affected the number of specimens that the field team were able to collect, such as willingness and ability of farmers to assist in restraining animals, the degree to which all of the animals in a village were assembled and weather conditions. A combination of these variables meant that a large number of the animals in some villages were not included in the study, producing a large difference between 'cattle sampled' and 'LC1 Estimate' for those villages.

When these villages are removed from the analysis of village herd size (n=33), the average 'cattle sampled' divided by the average 'LC1 Estimate' increases to 0.87 (191.47/219.66). Fig. 16 shows 'cattle sampled' plotted against 'LC1 estimate' when these outliers are removed. The R^2 value for the plot = 0.9014 indicating that 'cattle sampled' and 'LC1 estimate' are strongly related, and that there is little variation in the degree to which they are different.

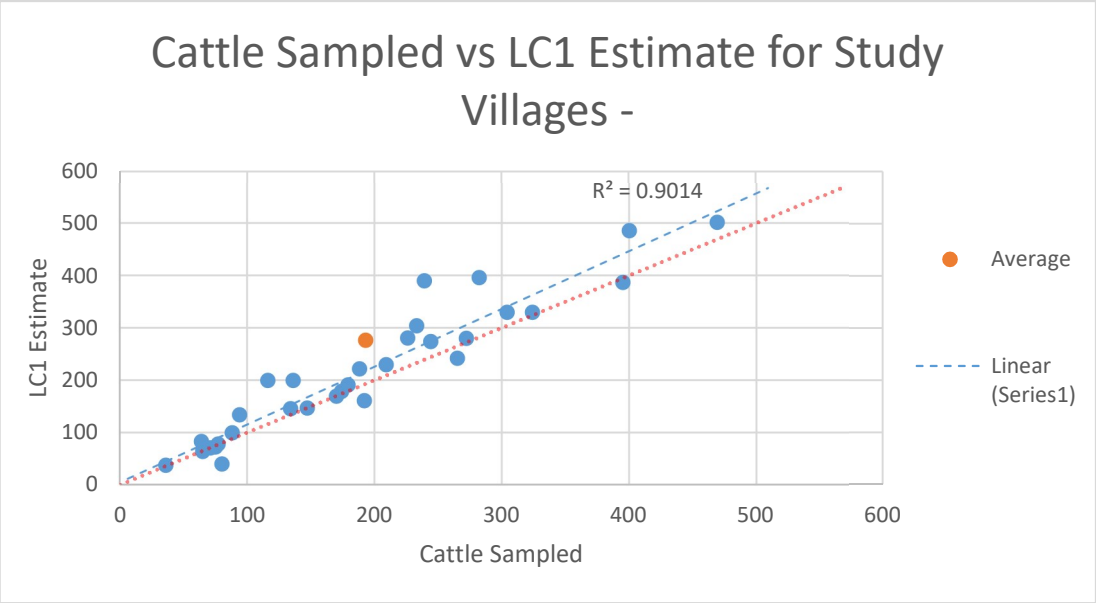

Figure 16. Scatter plot of 'cattle sampled' and 'LC1 estimate'

In order to determine if the two estimates of herd size vary significantly, first the normality of the distribution of the data was tested. Shapiro-Wilk Test was performed using both set of the measurements of herd size. The results indicated that the 'Cattle Sampled' data were normally distributed (p = 0.099) but that the 'LC1 Estimate' data were not (p = 0.002) (Table 8)

	Shapiro-Wilk Test		
	Statistic	d.f.	*p*
Cattle Sampled	0.951	38	0.099
LC1 Estimate	0.897	38	0.002

Table 8. Test for normality of distribution of herd size estimates

As the data were not both normally distributed, non-parametric testing to compare the means was necessary. The Mann-Whitney U Test was performed and the result (p=0.077 indicates that there is not a significant difference between the two estimates.

Agreement between the two estimates of herd size was tested using Spearman's ρ. Pearson's correlation coefficient was also calculated. The results indicate that the two estimates strongly agree (ρ=0.808; p =- <0.001) (Table 9).

	Spearman's ρ	*p*	Pearson's correlation coefficient	*p*
Cattle Sampled vs LC1 Estimate	0.808	.000	0.653	0.000

Table 9. Spearmans's ρ and Pearson's correlation coefficient of 'cattle sampled': 'LC1 estimate'

Although the means did not differ significantly and the measurements agreed, there was a difference between the two estimates for each village and a measurement of village herd size was required for use in analysis. It was assumed that the degree to which 'Cattle Sampled' and 'LC1 Estimate' varied from the true village herd size was consistent across all villages. It is not possible to know which measurement of herd size is more accurate, therefore, the figure used for village herd size henceforth was the mean of the two measurements (estimated village herd size (EVHS)).

$$\frac{Cattle\ Sampled + LC1\ Estimate}{2} = Estimated\ Village\ Herd\ Size$$

EVHS ranged from 37 in Entebbe village, Yumbe district to 573.5 in Pawinyo village, Yumbe district. The mean EVHS across the entire study area was 234.7 (SD = 136.61). Fig. 17 shows the distribution of EVHS across the study villages.

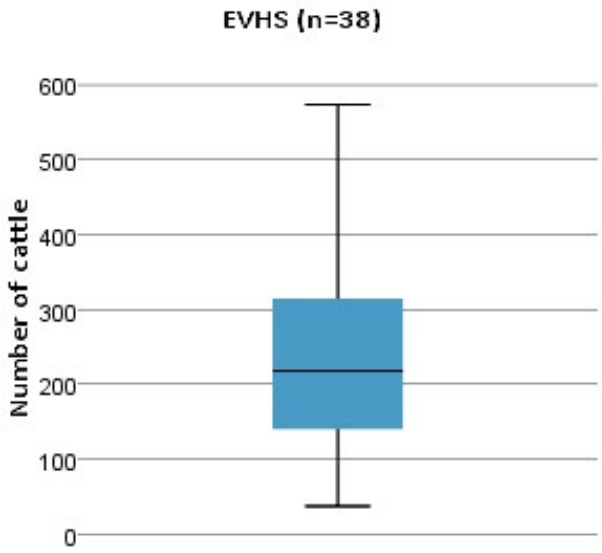

Figure 17. Box plot of EVHS values

3.2.2. Herd structure

Data were collected from 7406 cattle in 39 villages across 6 districts. There was an incomplete set of data collected for 22 of the individual animals due to either absent data or recording of inappropriate data e.g. recording 'F' under breed. These 22 individuals were excluded from analysis of herd structure, the remaining individuals (n=7384) were included. Table 10 shows the number of cattle belonging to each category of each characteristic. Frequencies are included for all animals for which data was recorded and for the subset that were screened with TBR-PCR. Odds ratios of belonging to each category were calculated for the entire sample and the animals screened with TBR-PCR to demonstrate that the subset is representative of the whole sample.

Sex						
		Female	Male			Total
Entire sample	Frequency	4960	2424			7384
	Percent	67.17%	32.83%			100.0%
Animals screened	Frequency	1638	806			2444
	Percent	67.02%	32.98%			100.0%
Odds Ratio (95% CI)		1.01 (0.91 – 1.11)	0.99 (0.90 – 1.09)			
Age						
		Calf	Juvenile	Adult		Total
Entire sample	Frequency	1077	1986	4321		7384
	Percent	14.59%	26.90%	58.52%		100.0%

Animals screened	Frequency	347	656	1441		2444
	Percent	14.20%	26.84%	58.96%		100.0%
Odds Ratio (95% CI)		1.03 (0.91 – 1.18)	1.00 (0.91 – 1.11)	0.98 (0.89 – 1.08)		
Breed						
		Ankole	Exotic	Mixed	Zebu	Total
Entire sample	Frequency	234	43	703	6404	7384
	Percent	3.17%	0.58%	9.52%	86.73%	100.0%
Animals screened	Frequency	84	9	223	2128	2444
	Percent	3.44%	0.37%	9.12%	87.07%	100.0%
Odds Ratio (95% CI)		0.92 (0.71 – 1.28)	1.58 (0.77 – 3.26)	1.05 (0.89 – 1.23)	0.97 (0.85 – 1.11)	
Body Condition Score						
		Lean	Medium	Fat		Total
Entire sample	Frequency	214	2870	4273		7384
	Percent	3.26%	38.87%	57.87%		100.0%
Animals screened	Frequency	78	911	1455		2444
	Percent	3.19%	37.27%	59.53%		100.0%
Odds Ratio (95% CI)		0.91 (0.70 – 1.18)	1.07 (0.97 – 1.18)	0.93 (0.85 – 1.02)		

Table 10. The frequency of individuals in each category for recorded characteristics.

3.2.2.1. Sex-Age Distribution

Overall, 67.17% (4960/7384) of the animals in the study were female and 32.83% (2424/7384) were male. At district level, this female-bias is present with the proportion of females varying from 49.94% (424/849) in Kitgum to 74.79% (2448/3273) in Yumbe. A female-bias was common across all six districts bar Kitgum in which 50.1% (425/849) of sampled animals were male (Fig.18).

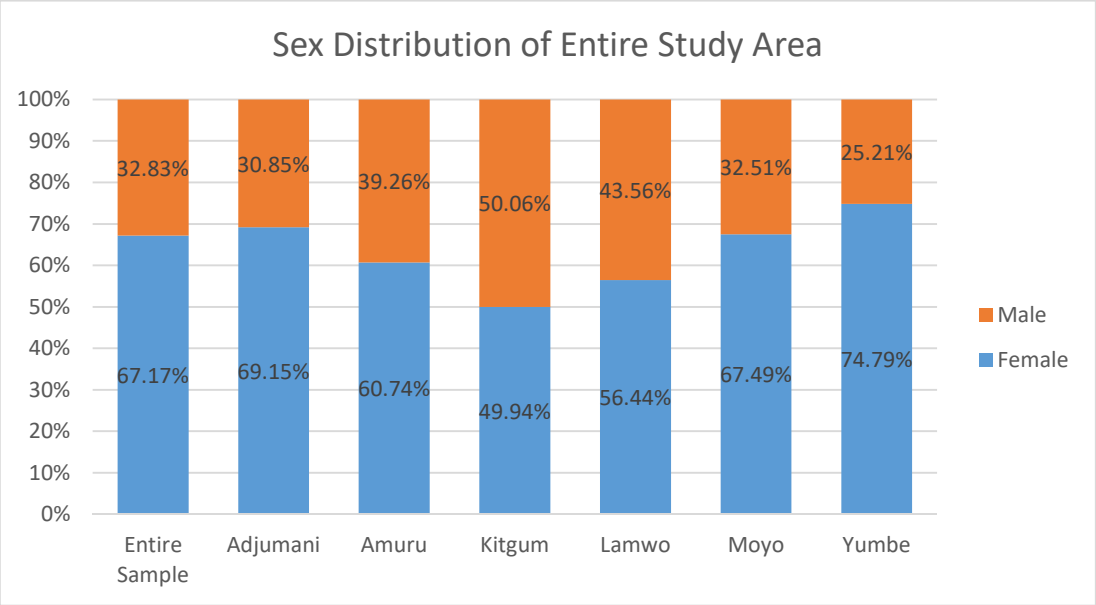

Figure 18. The proportion of animals of each sex in study districts

Of the entire sample, 58.52% (4321/7384) of animals were adults, 26.90% (1986/7384 were juveniles and 14.59% (1077/7384) were calves. This pattern of adults being the largest group, followed by juveniles was seen across all six districts (Fig. 19).

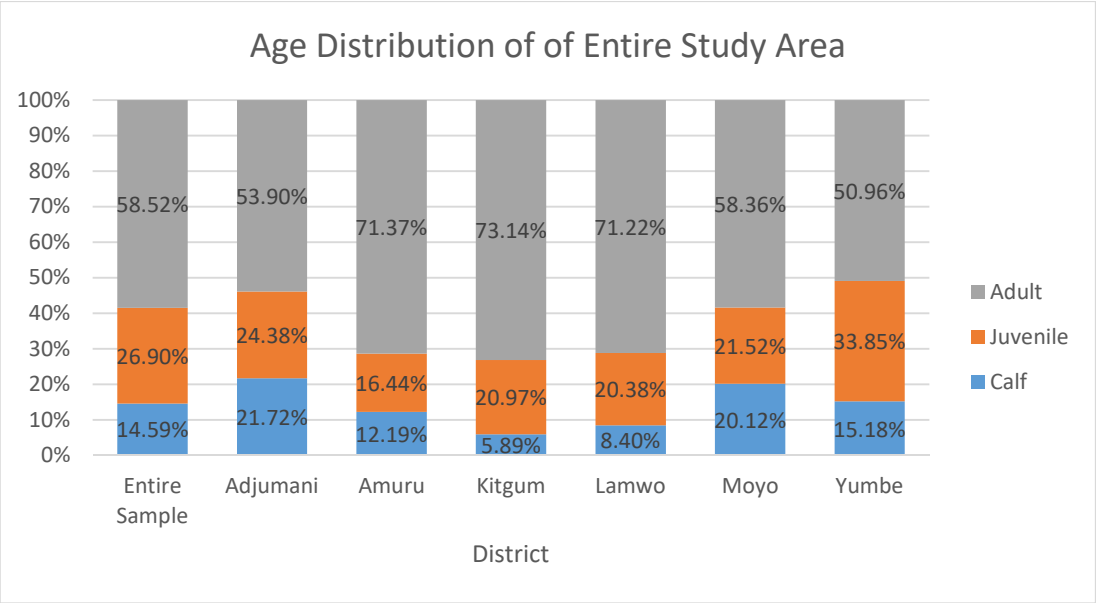

Figure 19. The distribution of age groups in the sample animals in each district.

The sex distribution became increasingly female-biased with advancing age of animals. Within calves, females only slightly in the majority at 51.81% (558/1077). Amongst juveniles we see a female-bias (56.24%; 1123/1993) and this is further increased in adults (76.0%; 3290/4331). Fig x shows the proportion of all cattle in each age-sex category and the proportion of male and female animals in each age group.

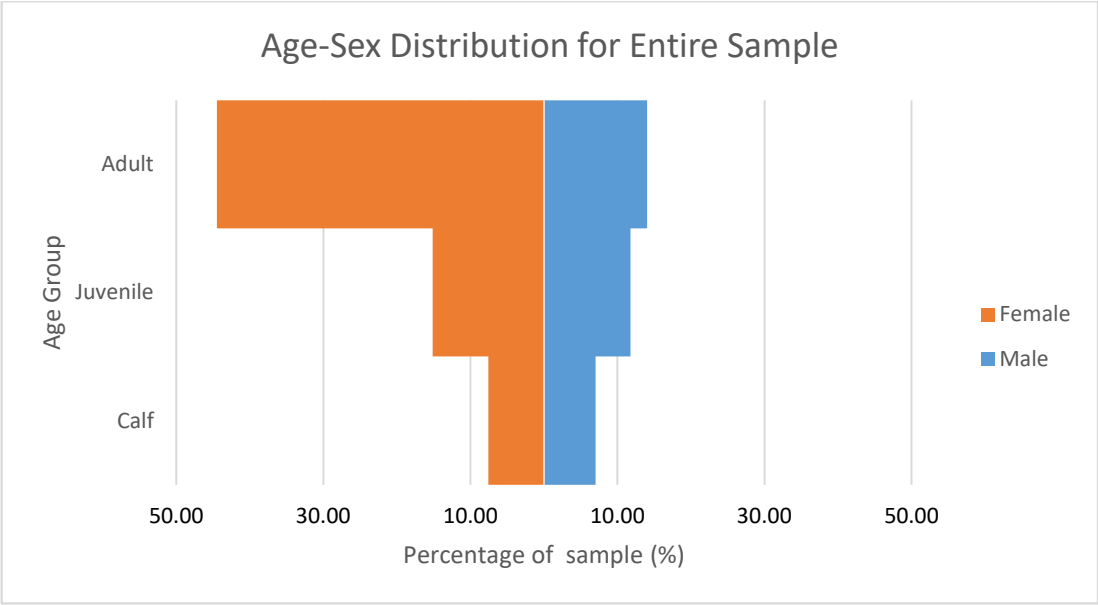

Figure 20. Age-sex pyramid for entire sample.

In Uganda, milk is the commodity for which cattle are mainly kept. (Government of Uganda, 2004) The Uganda country report (as part of SoW-AnGR produced by the FAO) (Government of Uganda, 2004) states this is in order to ensure a constant supply of milk. The report cites de Leeuw and Wilson (1987) who found that most African cattle owners manipulated herd structure to have a female-bias in order to maximise milk production.

With dairy cattle, milk production does not begin until the first calving. It therefore follows that, in a dairy production system, a large proportion of the herd will be mature females. This increasing female-bias as age increases indicates disproportionate off-take (sales minus purchases) of male cattle and/or acquisition of females.

Scoones (1990; cited in Chikura, 2006) found that off-take of cattle is low in communal grazing areas. He states that cattle are usually retained for draft power and manure in mixed-crop livestock systems and that most cattle sold are castrated males of advanced age which could no longer be used for draft power.

Further contributing to the female bias are NGOs, such us Heifer International that work in the provision of dairy cattle to areas as a means of supporting economic development. Part of the approach of Heifer International is ‘passing on the gift’; the concept of this being the provision of a female animal to a family who will then pass on the first female offspring of that animals back to the program. This calf will then be given to another family. (Birner et al, 2010) This process will further accentuate the female bias.

3.2.2.2. Breed

The breed of the animals included in the sample was identified as belonging to one of the four categories outlined in Table 11.

Zebu	**East African Shorthorn Zebu (*Bos indicus)* are indigenous to the study area. As seen in figure x. they have a small body frame with a large, muscular thoracic hump (Mbuza, 1995).**
Ankole	Ankole Longhorn (intermediate *Bos indicus* / *Bos Taurus)* have large bodies long legs and a small, musculo-fatty hump in the cervico-thoracic region (Mbuza, 1995). They have characteristic large, arched horns, as seen in fig x.
Exotic	Breeds not local to the area e.g. Friesian, Holstein. These, often European, breeds have been introduced to the area due to their superior milk and meat production over indigenous breeds. Problems associated with exotic breeds introduced to sub-Saharan African production systems are increased water requirements (King, 1983) and susceptibility to infectious disease (Murray et al, 2013) compared to locally adapted populations
Mixed	Cross breeds of Zebu, Ankole and exotic breeds. Farmers genetically improve their herds by breeding high yielding exotic breeds with indigenous animals (Mason and Buvanendran, 1982).

Table 11. Cattle breed data categories.

The vast majority of cattle in the sample were Zebu breed (86.73%; 6404/7384.) The district in which Zebu cattle made up the smallest majority was Amuru (55.51%; 287/517) and in Adjumani, Kitgum, Lamwo and Yumbe the proportion of animals of Zebu breed exceeded 85%.

The proportion of Ankole cattle also ranged widely. No Ankole cattle were included in the sample in Moyo district (0/646) and 0.25% and 0.52% of cattle in Adjumani (3/1206) and Yumbe (17/3273), respectively, were Ankole. Conversely, 14.78% (132/893) of cattle in Lamwo were Ankole, with Amuru also having a sizable proportion (10.06%; 52/517). It is worth noting that Ankole cattle have historically been found in the majority in central and western Uganda and that Amuru is the most south-westerly of the study districts. (Fig. 5).

Figure 21. (Left) East African Shorthorn Zebu
Figure 22. (Right) Ankole Longhorn

Exotic breeds largely made up a very small minority of animals at the district level with 0.58% (28/517) of the entire sample being exotic, however they were 5.42% (28/517) of cattle in Amuru.

The proportion of mixed breed cattle ranged widely between districts, from 29.01% (150/517) in Amuru to 4.64% (30/646) in Moyo, although this was consistently the second most common breed after Zebu (Fig. 23).

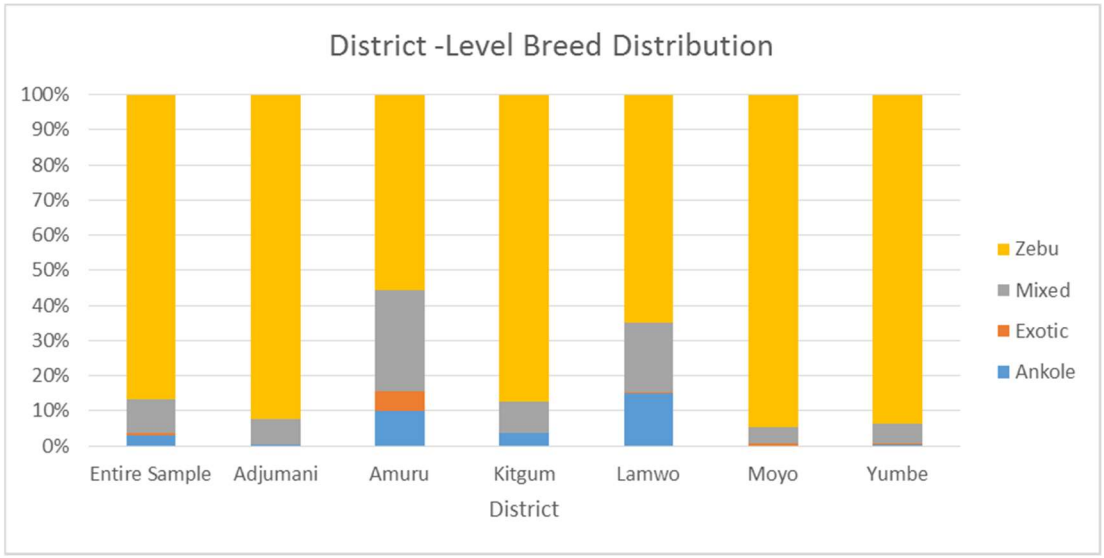

Figure 23. Breed distribution in study districts

Age and Breed

Programs such as those carried out by Heifer International (see Section 3.2.2.1) may go some way to explaining the greater proportion of adults within the exotic breed category in the sample, as it is high yielding exotic breeds that are often supplied. The larger proportion of cattle in the younger age groups that make up the Zebu category may be explained by the

dominance of this group of the entire sample, with Zebu cattle producing a greater number of offspring than the other breeds (Fig. 24). Farmers may be selling adult Zebus in order to raise capital and make available capacity for animals of Ankole and Exotic breeds.

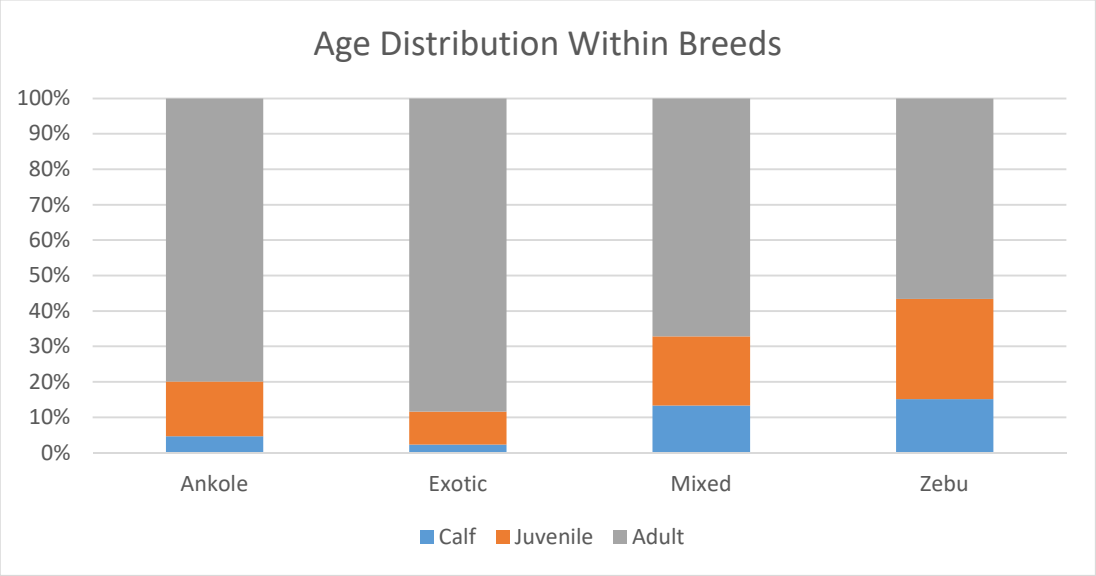

Figure 24. Age distribution within breed categories

3.2.2.3. Body Condition Score

An overwhelming proportion of the animals included were either fat or medium. In the entire sample, a majority of the animals were fat (57.87%; 4273/7384), 38.87% (2870/7384) were medium and only 3.26% (241/7384) were lean. In Amuru, 82.96% (429/517) of cattle were fat. The highest proportion of lean cattle was found in Yumbe 4.49%. (147/3273) compared to only 0.56% (5/893) of cattle in Lamwo (Fig. 25).

The high body condition scores of the animals are consistent with overall low prevalence of AAT although there are a panoply of other diseases of cattle in this area of which cachexia is a clinical manifestation. Unexpectedly, at district level Amuru has highest prevalence of *T. brucei* s.l. infection but this is the region with the highest body condition scores.

If body condition score can be considered as an indicator of lack of disease then the condition of the animals across the study indicates a lack of diseases other than just AAT.

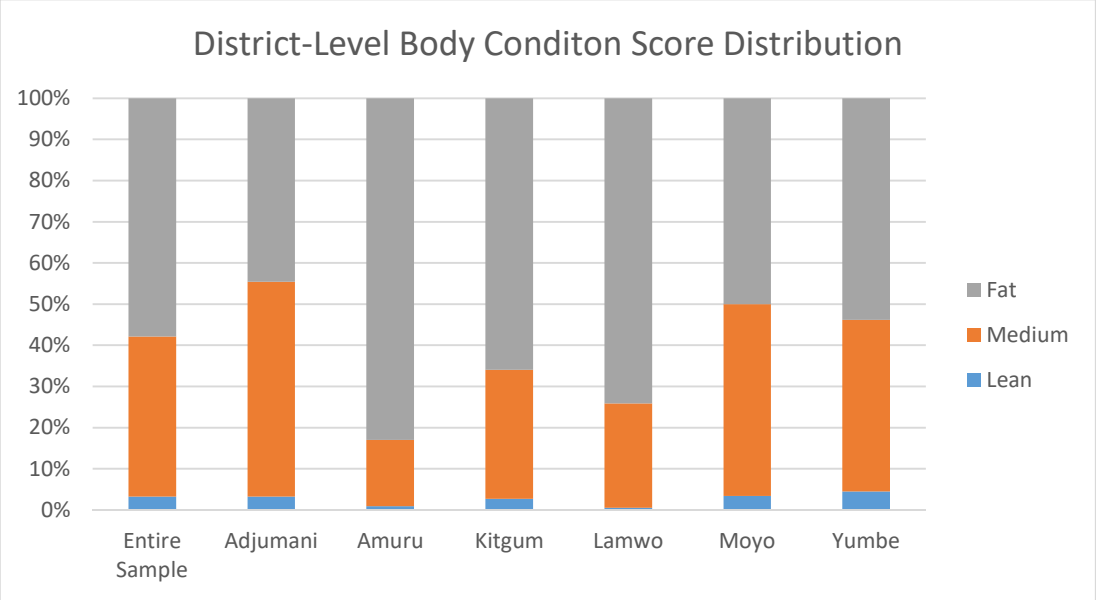

Figure 25. Body condition score of animals in study districts

3.2.3. Cattle Management

As with the analysis of herd structure (section 3.2.2.), data regarding cattle management were included in analysis for 7384 animals from 39 villages across 6 districts. Table 12 gives the frequency of cattle belonging to each category of each management characteristic. Frequencies are included for all animals for which data was recorded and for the subset that were screened with TBR-PCR. Odds ratios of belonging to each category were calculated for the entire sample and the animals screened with TBR-PCR to demonstrate that the subset is representative of the whole sample.

Traction Use Status		Used for traction	Not used for traction				Total
Entire sample	Frequency	740	6644				7384
	Percent	10.02%	89.98%				100.0%
Animals screened	Frequency	263	2181				2444
	Percent	10.76%	89.24%%				100.0%
Odds Ratio (95% CI)		0.92 (0.79 – 1.08)	1.08 (0.93 – 1.26)				
Grazing System		Communal	Communal and tethered	Tethered	Not grazed	Zero grazing	Total
Entire sample	Frequency	4344	2938	98	4	0	7384
	Percent	58.83%	39.79%	1.33%	0.05%	0.00%	100.0%
Animals screened	Frequency	1357	1048	36	3	0	2444
	Percent	55.52%	42.88%	1.47%	0.12%	0.00%	100.0%
Odds Ratio (95% CI)		1.14 (1.04 – 1.26)	0.88 (0.80 – 0.97)	0.90 (0.61 – 1.32)	0.44 (0.10 – 1.97)	N/A	

Table 12. The frequency of individuals in each category for recorded cattle management characteristics.

3.2.3.1. Traction use

In terms of the entire sample, the use of animals for traction is fairly uncommon with only 10.02% (740/7384) animals providing draft power. Of the animals used for traction, 96.22% (712/740 (Fig. 26) were adult males, the remainder were largely non-adult males (16/740), and females of all ages were only rarely used (12/740). As it is almost exclusively adult males that are used, this warrants particular attention to this group.

Yumbe district is unusual in that none of the animals are used for traction. All of the other districts utilise their adult male cattle for draft power by varying degrees. Farmers in Moyo only use 27.27% (21/77) of their adult males for traction. It is notable that the Moyo and Yumbe are both on the west of the Albert Nile whereas the other four districts are on the east side. The other four districts use between 68.73% and 83.17% of their adult males for traction. Kitgum is the district with the largest proportion of male cattle (see section 3.2.2.1) and is the district that uses the highest proportion of its adult males for draft power. The herd structure of this district and the use of adult males for traction make this the district with the greatest proportion of all cattle used for traction. This may represent a preference for mixed crop-livestock production over dairy production, unlike the other districts in the study which are maintaining female-biased herds.

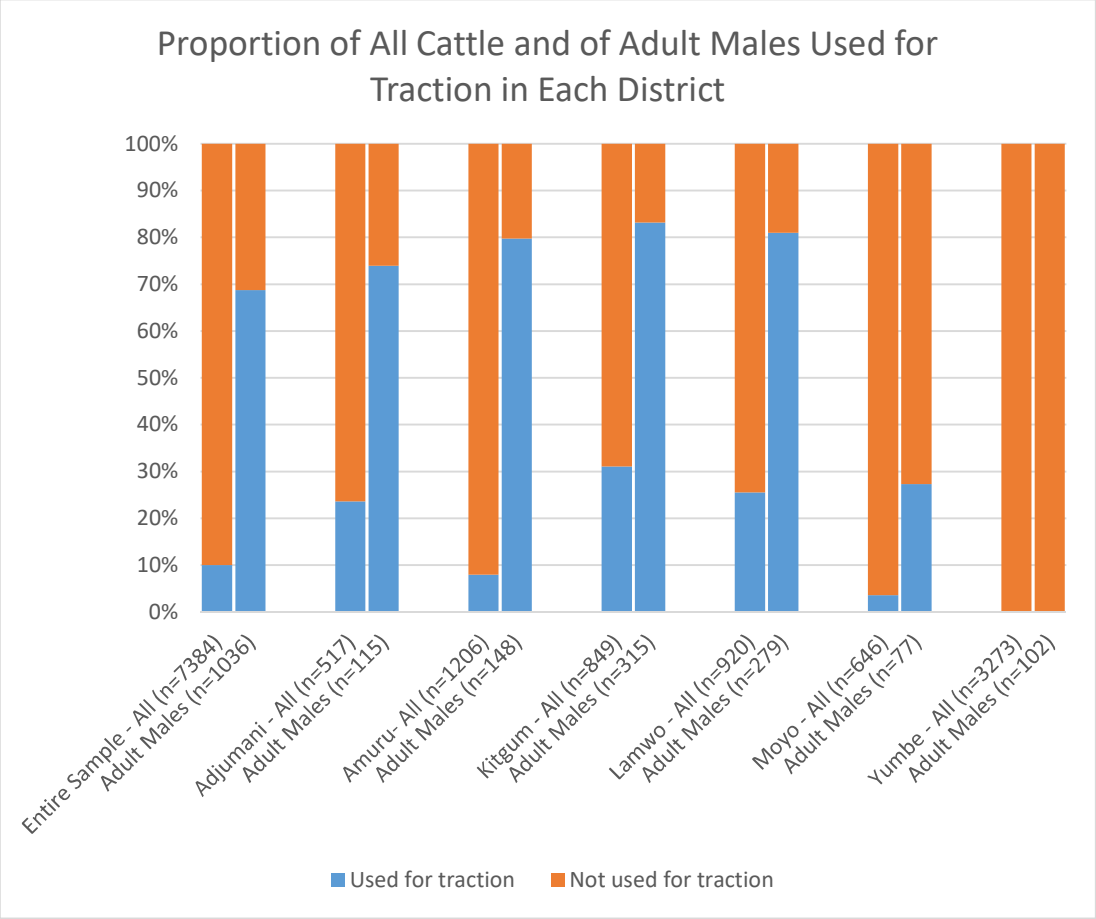

Figure 26. The proportion of all cattle and of adult males used for traction in each district.

Of all adult males, 68.73% (712/1036) were used for traction. This group only makes up 14.03% (1036/7384) of the entire sample (see Section 3.2.2.1.) but nevertheless, high prevalence of AAT, particularly in this group, would severely impact on mixed crop-livestock production if the ability of these animals to provide draft power were to be reduced. Okello et al (2015) found, in a study in south-eastern Uganda, that use of draft power is highly profitable but that this cattle output and household incomes were decreased by AAT due to reduced work capacity of diseased animals. The impact of AAT in animals used for traction also has wider effects in the community as diseased animals are unsuitable, due to ill health, for hire by others. Required input of human labour into ploughing is increased and crop yields are potentially reduced, impacting on food security, due to delays in ploughing.

3.2.3.2. Grazing system

A number of different grazing systems are in use by the cattle farmers in the study area. The most common system used is communal grazing (58.83%; 4344/7384) (Fig. 27). This system

utilises commonly owned pastoral land for extensive cattle production. This practise is discouraged, (Wozemba and Nsanja, 2008; cited by Ekou, 2014) but is still used in Northern Uganda due to historical traditions of pastoralism in the area.

A further 39.79% (2938/7384) of cattle were grazed communally in conjunction with tethered grazing. In a tethered grazing system, animals are tethered while grazing, unable to roam freely. Tethered grazing mostly occurs off-farm, making areas of land available for crop production that would otherwise be grazed. Animals that are sick or pregnant may be tethered while grazing. Only 1.33% (98/7384) of animals were managed by solely tethered grazing.

No animals were managed using zero grazing. In this more intensive system, water and feed are brought to the animals that are kept in small enclosures (Wozemba and Nsanja, 2008; cited by Ekou, 2014).

It is notable that a very small proportion of animals were not grazed (0.05%; 4/7384). Animals not grazed were all in the calf age group. It follows that the animals not grazed are suckling calves.

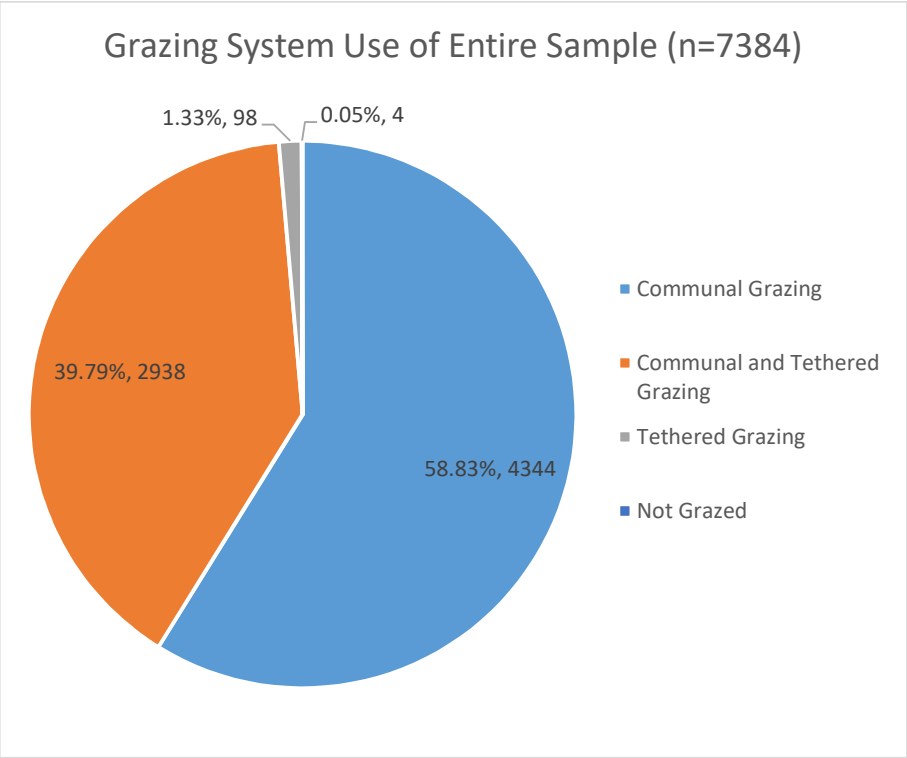

Figure 27. Grazing system of entire cattle sample

Communally grazed, and cattle grazed communally and while tethered, invariably make up the vast majority of the cattle in the sample. In Adjumani, 98.92% (1193/1206) of cattle were communally grazed and 98.00% (832/849) in Kitgum. In Moyo and Yumbe the proportion of animals crazed both communally and while tethered is 68.73% (444/646) and 33.39% (2173/3273) respectively. There was also variation between districts with regards to other grazing systems. Of the 98 study animals grazed exclusively while tethered, 78 of these animals were found in Amuru district. This is the only district with a sizeable number of animals grazed in this system (Fig. 28).

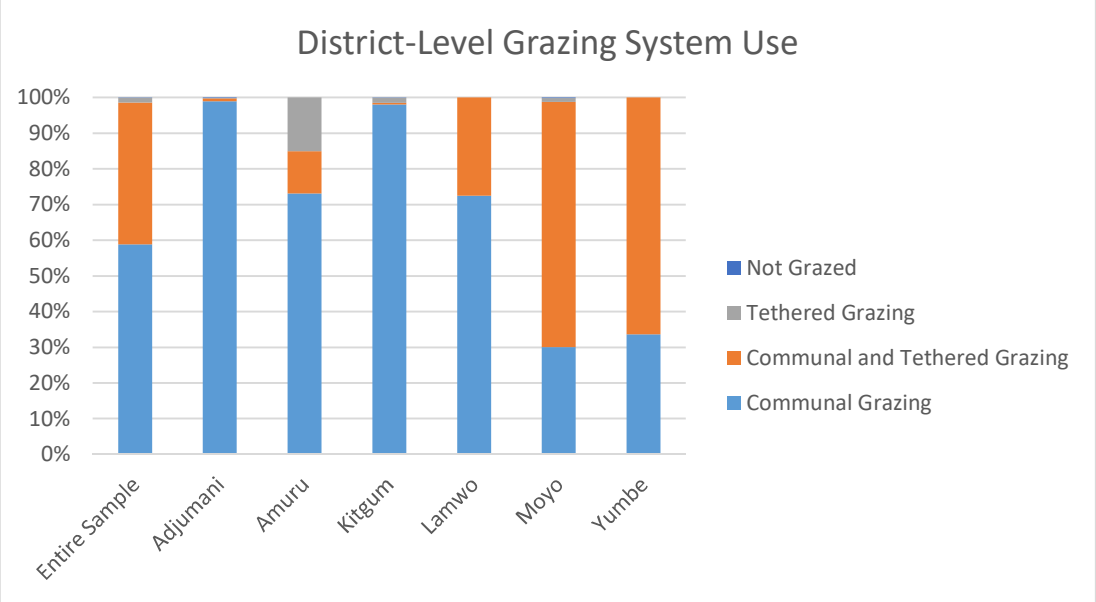

Figure 28. Grazing system of cattle in study districts

Of the body condition score groups that make up the grazing system group, a majority were fat in all grazing systems apart from 'not grazed. There appear to be differences in the body condition score, breed and age makeup of the 'not grazed' category but it must be borne in mind that only 4 animals in this grazing group were included in the sample and thus nothing can be drawn from the observations of this group.

The proportion of fat animals in the tethered grazing category was higher than other grazing systems. This may be influenced by pregnant animals being managed by tethered grazing. The practice of not allowing animals to roam freely may also reduce the level of tick challenge faced by cattle, resulting in lower prevalence of cachexic tick-borne diseases and causing cattle grazed this way to have higher body condition scores (Fig. 29).

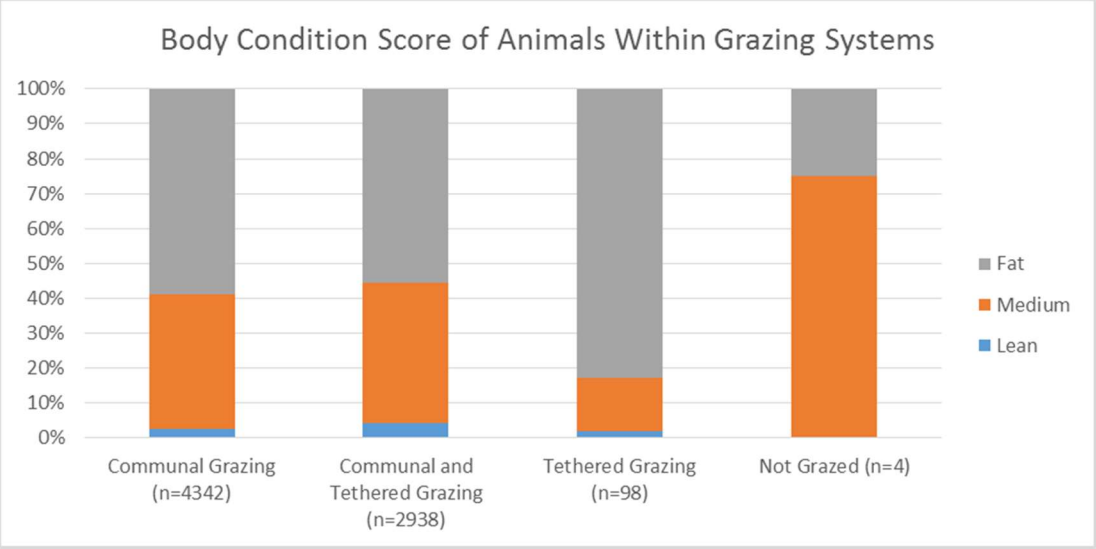

Figure 29. Body condition score of animals within each grazing system category

What is notable when looking at breed and grazing system, is the increased proportion of 'tethered grazing' animals of the Ankole group (Fig. 30). This may be due to the fact that Ankole are more valuable, both in terms of monetary value and their higher production of milk and draft power output (Magona et al, 2011), and this may result in farmers taking extra measures to safeguard the health of these animals i.e. managing them through tethered grazing as opposed to communal grazing.

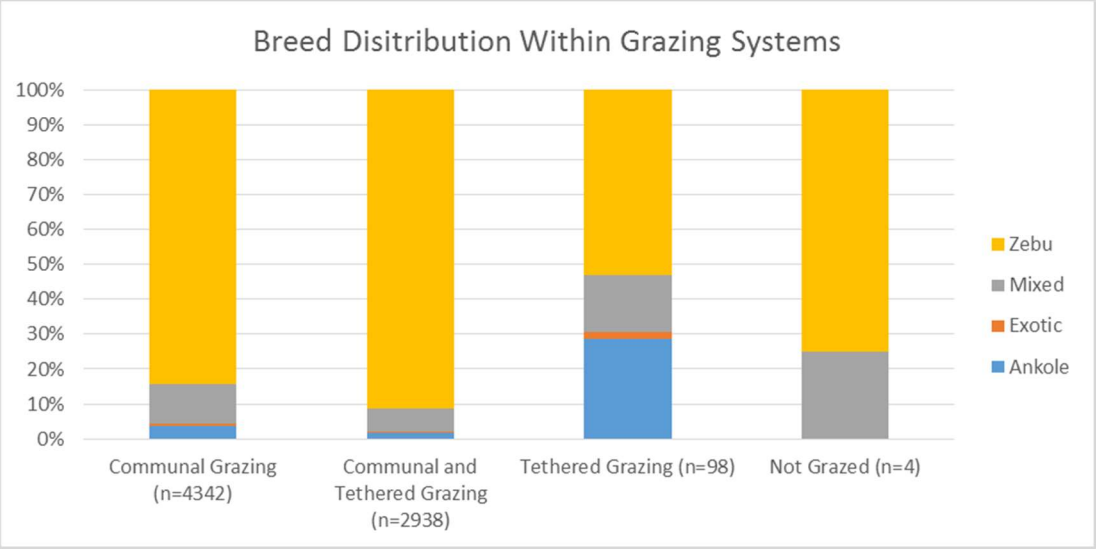

Figure 30. Distribution of breeds within grazing system categories

3.3. Results of TBR-PCR Screening

Of the 7406 blood specimens collected from individual animals across the entire study area, 2463 were screened with TBR-PCR. This represents 25% of the specimens collected from

each village; screening at least 50 or all if less than 50 specimens were collected. Tables 10 and 12 shows that the screened cattle are representative of the entire sample with regards to herd structure. In order to verify that the animals screened with TBR-PRC were representative of the entire sample, odds ratios were calculated for each category e.g. 'Ankole : other', 'Fat: other'. The ratios for all categories were statistically insignificant (the upper bound of the 95% CI was greater than one and the lower bound was less than 1) for all categories except 'Communal : other' and 'communal and tethered : other' . This indicates that for sex, age, breed, body condition score and traction use status, the specimens screened were a representative sample of the entire study sample. Within grazing systems, a slightly smaller proportion of the animals screened were communally grazed (55.52%; 1357/2444) than in the entire sample (58.83%; 4344/7384). The proportion of screened animals grazed communally and tethered was slightly larger (42.88%; 1048/2444) than of the entire sample (39.79%; 2938/7384). Animals were only lost and gained to a significant degree between these groups.

3.3.1. Prevalence

Overall, the prevalence of *Trypanozoon* positive results from TBR-PCR screening was 2.61% (64/2463; 95% CI = 1.94% – 3.19%) for the entire study area. The mean average village prevalence was 2.81% (SD = 5.15%)

3.3.2. District

Wide variability in risk of trypanosome infection was found between animals in the six study districts. Prevalence ranged from 14.69% (21/153; 95% CI = 9.74% – 21.48%) in Amuru district to 0.73% (7/957; 95% CI = 0.32% – 1.53%) in Yumbe (Fig. 31).

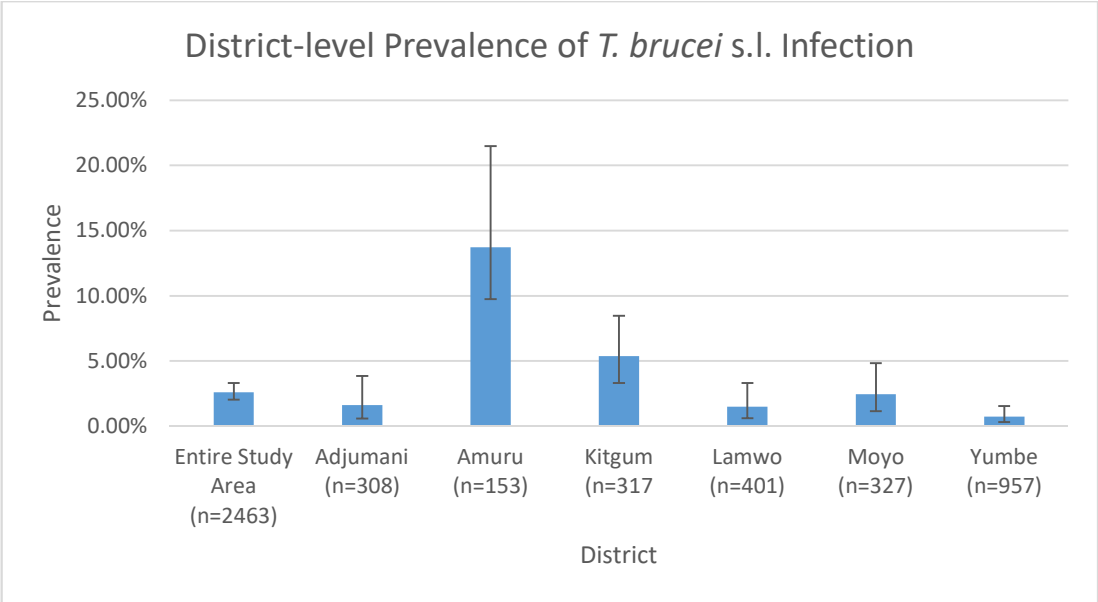

Figure 31. District-level prevalence of T. brucei s.l. infection

Animals in Yumbe (Yumbe : Other; OR = 0.19, p = <0.001) were significantly less likely to be infected with trypanosomiasis than animals in other districts. This district is the furthest to the north west of all of the districts and is separated from the other five study districts and from the higher prevalence areas to the south of Uganda by the White Nile (see Fig. 5). The lower risk of AAT infection in the cattle of this district may be attributed to their distance and restricted transport routes from higher prevalence areas.

Cattle in Kitgum district were 2.11 times more likely to be infected with trypanosomes than other districts (Kitgum : Other; OR = 2.11, p = 0.012) and the risk of infection of cattle in Amuru was severely increased (Amuru : Other; OR = 9.12, p = 0.001) (Table 13). Amuru is the district in this study furthest to the south towards higher prevalence areas. Another factor in the increased risk of infection in this is that Amuru district (then part of a larger Gulu district) was one of the districts worst affected districts by the LRA insurgency, this may have cause the structures necessary to control AAT unable to do so. Differences in risk of other districts were not statistically significant.

District	n	Number of TBR-PCR positives	Pairing	*p* - value	Odds ratio
Adjumani	308	5	Adjumani : Other	0.242	0.58 (0.23 – 1.46)
Amuru	142	21	Amuru : Other	<0.001[F]*	9.12 (5.25 – 15.85)
Kitgum	317	15	Kitgum : Other	0.012*	2.11 (1.17 – 3.80)
Lamwo	400	8	Lamwo : Other	0.397	0.72 (0.34 – 1.53)
Moyo	325	8	Moyo : Other	0.849	0.93 (0.44 – 1.97)
Yumbe	952	7	Yumbe : Other	<0.001	0.19 (0.08 – 0.41)
Total	2444	64			

Table 13. Odds ratios of T. brucei s.l. infection between study districts

Villages across All Districts

Of the thirty nine villages in the study, seventeen contained specimens which returned positive results from TBR-PCR screening. Each of the six districts in the study area contained at least one of these villages. Prevalence varied significantly between these villages (Fig. 32 shows the locations of the study villages and the prevalence of *T. brucei* s.l. infection found in each village. Prevalence ranged from 1.02% in Paubu, Moyo District (1/98; 95% CI = 0.10% – 6.11%) to 10% in Mirieyi-1, Adjumani District (5/50; 95% CI = 3.91% – 21.79%) and Bardyang, Kitgum District (5/50; 95% CI = 3.91% – 21.79%). In Teddi village in Amuru district, prevalence was 28.00% (15/50; 95% CI = 14.16% – 37.55%). This was significantly higher than all villages, ('Teddi : Other', OR = 20.95, 95% CI = 10.74 - 40.84) and then the villages with the next highest prevalence (Teddi : Miriey-1, OR = 3.86, 95% CI = 1.28 - 11.64).

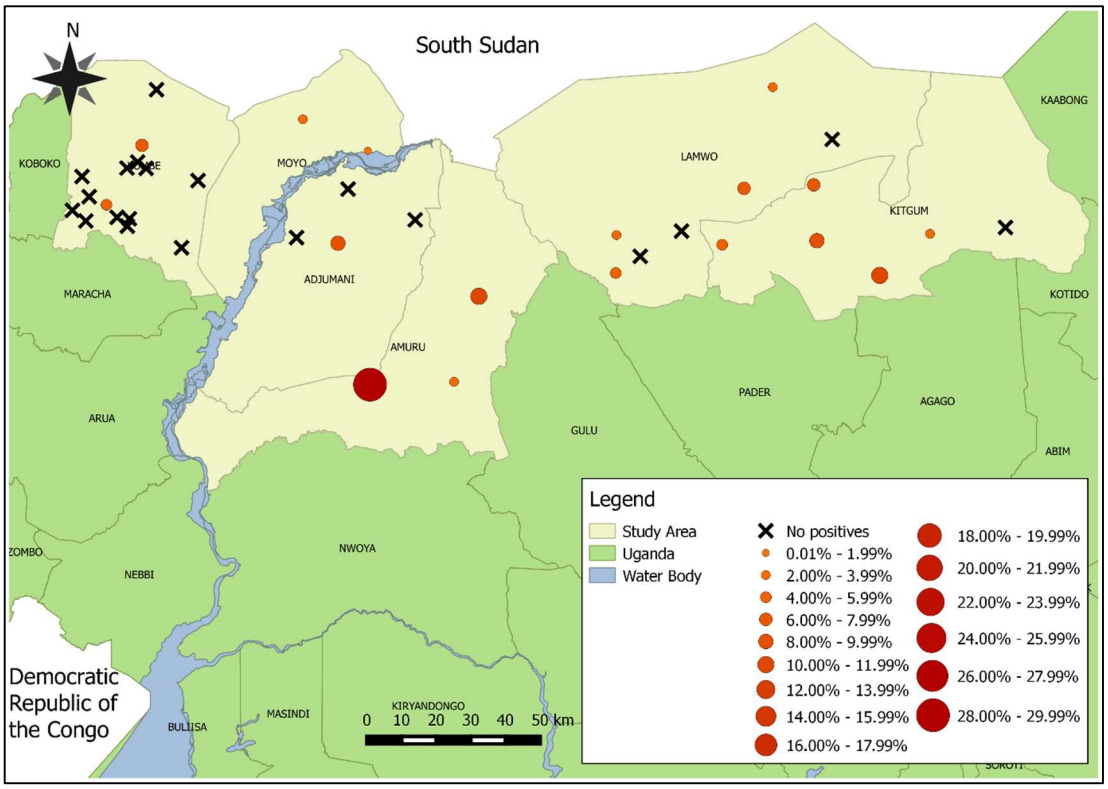

Figure 32. Prevalence of T. brucei s.l. infection found in study villages.

Correlation between EVHS and T. brucei *s.l. Prevalence*

The Pearson's correlation Coefficient was calculated between EVHS and *T. brucei* s.l. prevalence. The correlation was 0.084 and was statistically insignificant (p=0.614). Spearman's rho was calculated at 0.162 and was also statistically insignificant (p=0.330).

Adjumani

Of the five villages in Adjumani district, only specimens from the village Mirieyi-1 were *Trypanozoon* positive. The prevalence in this village was 10.00% (5/50; 95% CI = 3.91% – 21.79%). All specimens from the four remaining villages in Adjumani (Forohwa, Pawinyo, Mazaangwa and Asisi) were *Trypanozoon negative.* The mean average prevalence of villages in Adjumani district was 2.00% (SD = 4.47%).

Amuru

Specimens from all three of the villages in Amuru district were *Trypanozoon* positive. Pakuma village had the lowest prevalence at 3.77% (2/53; 95% CI = 0.31% – 13.49%). Teddi village had the highest prevalence, and the highest of all villages in the study, at 28% (15/50; 95% CI = 14.16% – 37.55%). The mean average prevalence of villages in Amuru district was 13.26% (SD = 12.94%). Fig. 33 shows the prevalence in each village in Amuru.

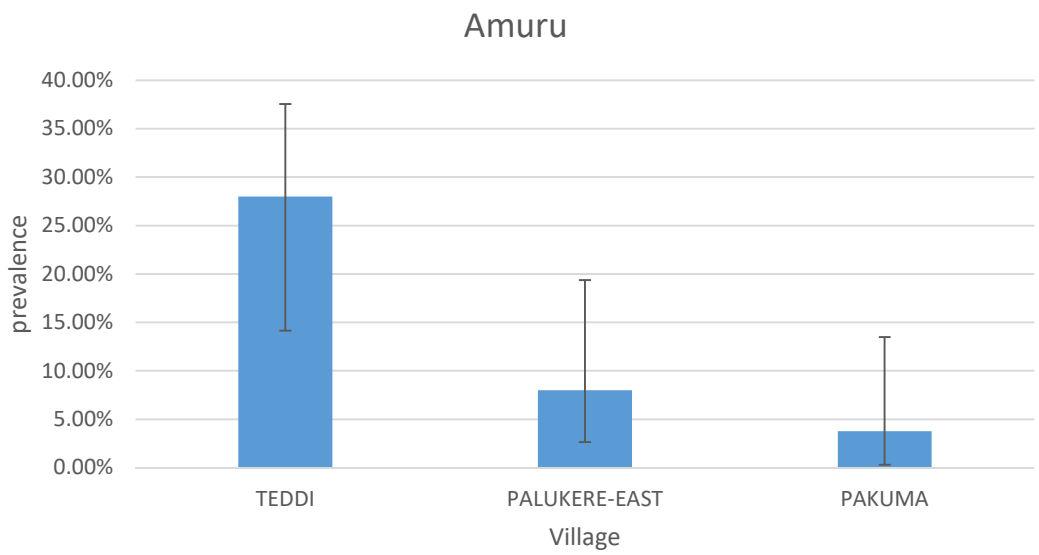

Figure 33. Prevalence of T. brucei s.l. infection in villages in Amuru district. Error bars represent the 95% CI.

Kitgum

Five of the six villages in Kitgum district had *Trypanozoon* positive specimens. The highest village prevalence was in Bardyang at 10% (5/50; 95% CI = 3.91% – 21.79%). The lowest prevalence of the villages with *Trypanozoon* positive samples was in Ladwogi at 2.00% (1/50; 95% CI = 0.10% – 11.47%). Madi Opei was the only village to have zero prevalence. The mean average prevalence of villages in Kitgum district was 5.00% (SD = 3.74%). Fig. 34 shows the prevalence in each village in Kitgum.

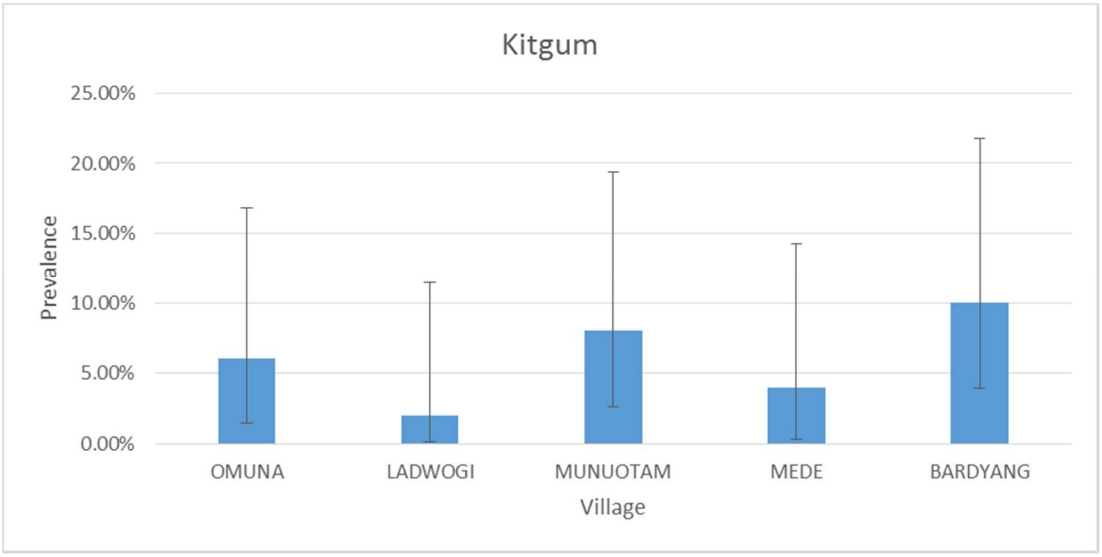

Figure 34. . Prevalence of T. brucei s.l. infection in villages in Kitgum district. Error bars represent the 95% CI.

Lamwo

Of the seven study villages in Lamwo district, four contained *Trypanozoon* positive cattle. The village with the highest prevalence was Kamama at 6.00% (3/50; 95% CI = 1.44% – 16.84%). Larach Odong was the village with the lowest prevalence above zero at 2.00% (1/50; 95% CI = 0.10% – 11.47%).

The mean average prevalence of villages in Lamwo district was 2.08% (SD = 2.33%). Fig. 35 shows the prevalence in each village in Lamwo.

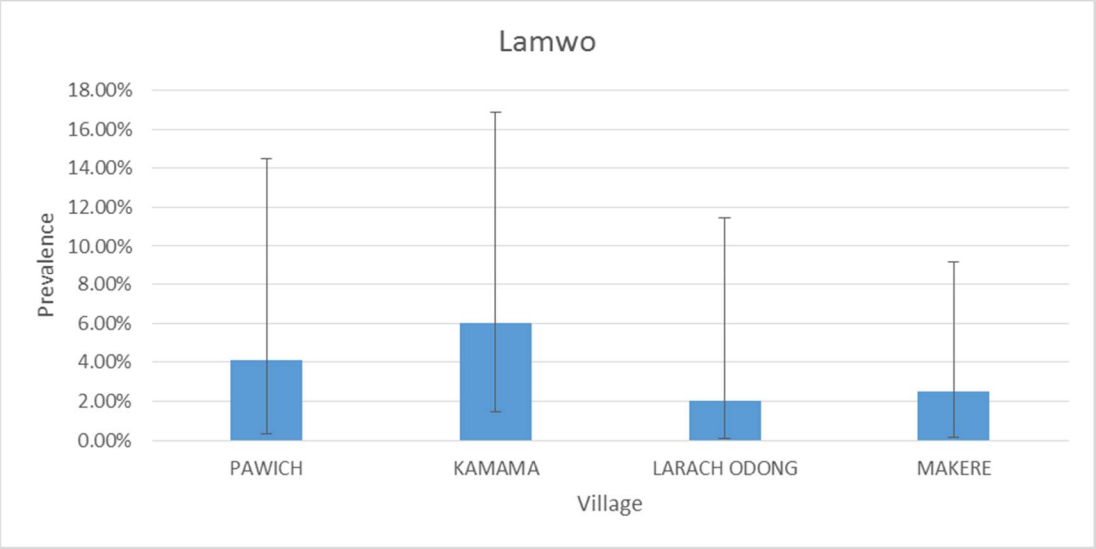

Figure 35. Prevalence of T. brucei s.l. infection in villages in Lamwo district. Error bars represent the 95% CI.

Moyo

Two of the three villages in Moyo district were found to have *Trypanozoon* positive specimens. The prevalence in Toloro village was 3.91% (7/179; 95% CI = 1.77% – 8.05%). The prevalence in Paubu village was 1.02% (1/98; 95% CI = 0.10% – 6.11%). None of the samples from Kuleni village were *Trypanozoon* positive. The mean average prevalence of villages in Moyo district was 2.47% (SD = 2.04%). Fig. 36 shows the prevalence in each village in Moyo.

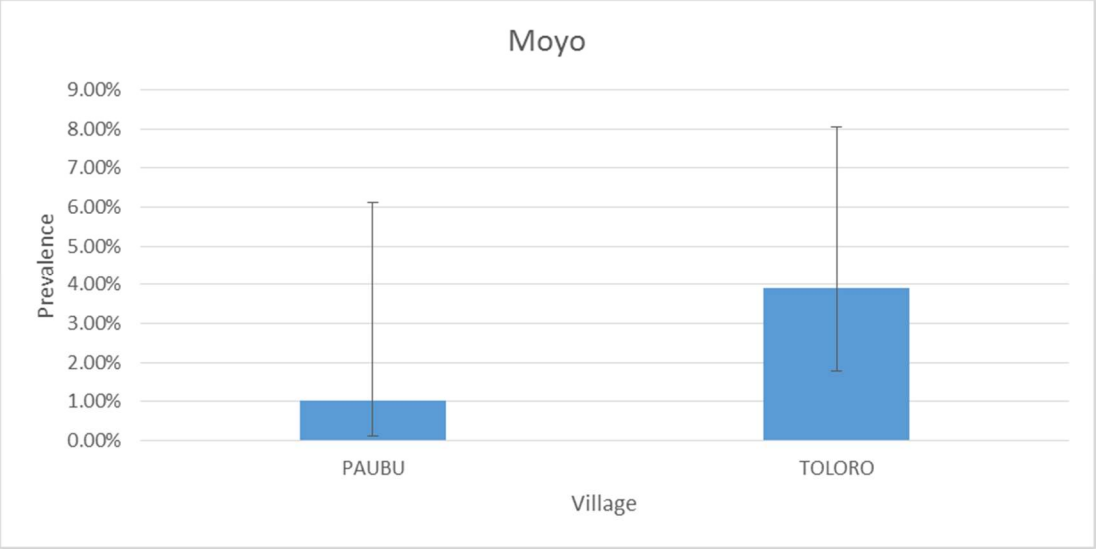

Figure 36. Prevalence of T. brucei s.l. infection in villages in Moyo district. Error bars represent the 95% CI.

Yumbe

Of the fifteen villages in Yumbe district, only samples from Wabanga and Paduru villages were found to be *Trypanozoon* positive. In Wabanga the prevalence was 6.17% (5/81; 95% CI = 2.33% – 13.98%) and in Paduru the prevalence was 4.00% (2/50; 95% CI = 0.34% – 14.22%). The mean average prevalence of villages in Yumbe district was 0.68% (SD = 1.84%). Fig. 37 shows the prevalence in each village in Yumbe.

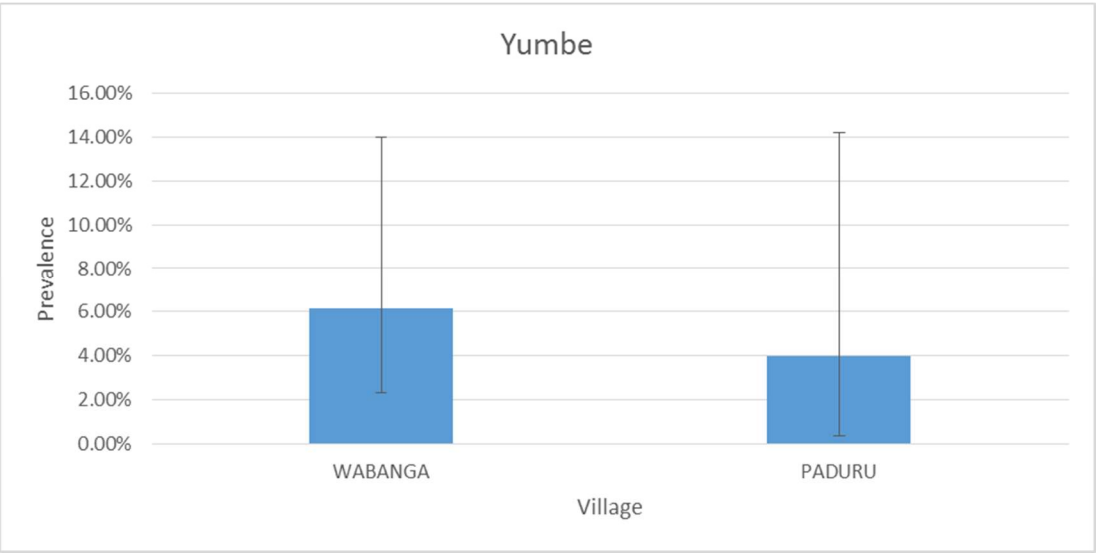

Figure 37. Prevalence of T. brucei s.l. infection in villages in Yumbe district. Error bars represent the 95% CI.

3.4. Risk Factor Analysis

Chi-square tests were performed to determine if there were associations between recorded characteristic (district, sex, age, breed, body condition score, traction use status and grazing system) and *T. brucei* s.l. infection in the animals screened with TBR-PCR. The analysis was carried out at the level of the entire study area as the number of TBR-PCR positive specimens was too low to perform meaningful this analysis at the district level or the village level.

A *p* – value less than 0.05 was considered to represent a statistically significant association. For tests where an outcome (*infection* or *lack of infection*) had an expected value of less than 5, Fisher's Exact Test was performed instead of Pearson's chi-square. This is indicated by [F] alongside the *p* – value in the results.

Odds ratios were calculated for each for each category. Statistically significant *p* – values (*p* = <0.05) are indicated by * in the table. Odds ratios (OR) are considered significant if:

- OR greater than 1 with a lower bound of 95% CI greater than 1
- OR less than 1 with upper bound of 95% CI less than 1

Significant odds ratios are indicated by * in the table.

3.4.1. Sex

The Pearson's Chi-square test indicated no significant association between sex and *T. brucei* s.l. infection ($p = 0.610$). The female : male odds ratio (OR=1.44; $p = 0.610$) was also insignificant.

Sex	n	Number of TBR-PCR positives	Pairing	*p* - value	Odds ratio (95% CI)
Female	1638	41	Female : Male	0.610	1.14 (0.68 – 1.92)
Male	806	23			
Total	2444	64			

Table 14. Odds ratio between male : female animals for T. brucei s.l. infection

3.4.2. Age

There was no significant risk of infection for calves compared to other groups. Juveniles were 0.33 ($p = 0.004$) times as likely to be infected as other age groups and adults were 2.13 ($p = 0.008$) times as likely to be infected.

This increased risk of infection in adults compared to juveniles have a number of contributing factors. Torr and Mangwiro (1998) found that the probability of younger

animals being bitten was lower than that of adults, with juveniles performing defensive leg movements at a higher rate which correlated inversely with feeding success rate of flies. They found that only ≈10% of tsetse fed on 12 month old calves compared to ≈50%-60% on adults.

Rowlands et al (1993) found that prevalence of AAT increased with age and attributed this to increased exposure to the vector with age and cumulative exposure to multiple trypanosome infections, incompletely eliminated by treatments.

Age Group	n	Number of TBR-PCR positives	Pairing	*p* - value	Odds ratio (95% CI)
Calf	347	9	Calf : Other	0.975	0.99 (0.43 – 2.04)
Juvenile	656	7	Juvenile : Other	0.004*	0.33 (0.13 – 0.72)*
Adult	1441	48	Adult : Other	0.008*	2.13 (1.20 – 3.76)*
Total	2444	64			

Table 15. Odds ratios between age categories for T. brucei s.l. infection

Fig. 38 shows the difference in age composition of animals screened with TBR-PCR and the subset those of animals infected with *T. brucei* s.l. The striking feature is the dominance of the infected cattle subset by adult animals.

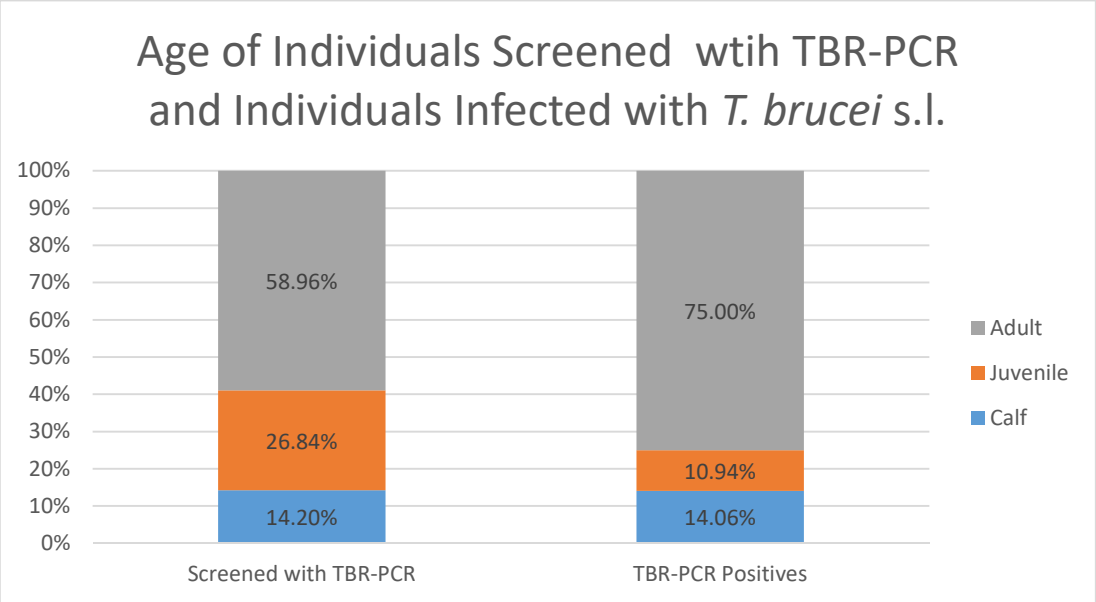

Figure 38. The proportion of animals screened with TBR-PCR and animals infected with T. brucei s.l. in each age category

3.4.3. Breed

Zebu cattle were the breed with the lowest risk of AAT infection in the study. The risk of infection in zebu was significantly lower than in Ankole (Zebu : Ankole; OR = 0.36, p = 0.04) and in mixed breeds (Zebu : mixed; OR = 0.44, p = 0.013). The difference in risk of infection between Zebu and Exotic cattle was not statistically significant.

This increased risk of detectable trypanosome infection in Ankole cattle is in agreement with findings of Magona et al (2004) who studied difference prevalence of trypanosome infection between Zebu and Ankole cattle in northern Uganda.

Outbreeding depression occurs with regards to vulnerability to infectious disease and this may be a factor in the higher risk of infection for mixed breed cattle. Murray et al (2013), in a general study of infectious disease in East African Shorthorn Zebu, found a positive association between clinical illness and genetic introgression from exotic breeds.

Breed	n	Number of TBR-PCR positives	Pairing	p - value	Odds ratio
Ankole	84	5	Ankole : other	0.665[F]	2.47 (0.96 – 6.32)
Exotic	9	1	Exotic : other	0.2128[F]	4.71 (0.58 – 38.20)
Mixed	223	11	Mixed : other	0.023*	2.12 (1.09 – 4.13)*
Zebu	2128	47	Zebu : other	0.001*	0.40 (0.23 – 0.70)*
			Zebu : Ankole	0.0449*	0.36 (0.14 – 1.24)*
			Zebu : Mixed	0.013*	0.44 (0.22 – 0.95)*
			Zebu : Exotic	0.1852[F]	0.18 (0.02 – 1.47)
Total	2444	64			

Table 16. Odds ratios between breed categories for T. brucei s.l. infection

Fig. 39 shows the difference in breed composition of animals screened with TBR-PCR and the subset those of animals infected with *T. brucei* s.l. The chart clearly shows that a disproportionate number of mixed breed animals were found to be infected (OR = 2.12; p = 0.023)

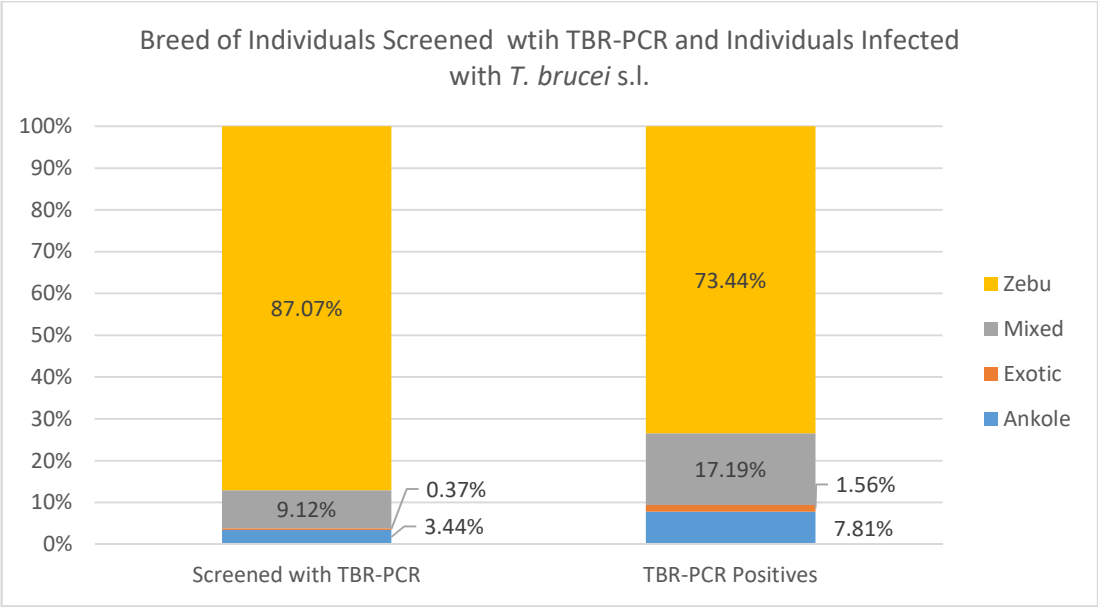

Figure 39. The proportion of animals screened with TBR-PCR and animals infected with T. brucei s.l. in each breed category.

3.4.4. Body Condition Score

No tests indicated significant association between body condition score and *T. brucei* s.l. infection. This is surprising as cachexia is a cardinal symptom of AAT in cattle. This result may be due to the fact that there are a panoply of conditions causing cachexia and reduced appetite that affect the cattle of this region e.g. theileriosis, bovine anaplasmosis.

Wastage of cattle and decreased meat production are an economic threat to cattle owners. Many of the infections that manifest in this way are tick-borne and this may go some way, among other factors, to explaining the insecticide products used by farmers in this sample (see section x.x – insecticide spraying).

Body Condition Score	n	Number of TBR-PCR positives	Paring	*p* - value	Odds ratio
Lean	78	1	Lean : other	0.7216 [F]	0.47 (0.06 – 3.47)
Medium	911	18	Medium : other	0.125	0.65 (0.38 – 1.13)
Fat	1455	45	Fat : other	0.075	1.63 (0.95 – 2.80)
Total	2444	64			

Table 17. Odds ratios between age groups for T. brucei s.l. infection.

3.4.5. Traction Use Status

All Animals Screened with TBR-PCR

The Pearson's chi square test for association between traction use status *T. brucei* s.l. infection indicated a significant association (p=0.037). The odds ratio was 0.51 (not used for traction : used for traction) and this was statistically significant.

Traction use	n	Number of TBR-PCR positives	Pairing	*P* - value	Odds ratio (95% CI)
Not used for traction	2181	52	Not used : Used	0.037*	0.51 (0.27 – 0.97)*
Used for traction	263	12			
Total	2444	64			

Table 18.Odds ratio for the entire sample of animals used for traction : animals not used for traction for T. brucei s.l. infection

Adult Males

As discussed in Section 3.2.3.1., traction use is limited almost exclusively to adult males. Including the entire sample in the analysis of association between *T. brucei* infection and traction use would misleadingly inflate the number of animals not used for traction. This analysis therefore only considers adult males in order to produce an accurate measurement of association.

When only adult males are included in the analysis, neither the Fisher's Exact Test (p = .0.4074) nor odds ratio are statistically significant (OR = 0.47; 95% CI = 0.06– 3.47).

Traction use (Adult Males)	n	Number of TBR-PCR positives	Pairing	*p* - value	Odds ratio
Not used for traction	115	3	Not used : Used	0.4074 [F]	0.47 (0.06 – 3.47)
Used for traction	252	12			
Total	367	15			

Table 19. Odds ratio for adult males used for traction : not used for traction for T. brucei s.l. infection

3.4.6. Grazing System

Cattle managed in the tethered grazing system were at extremely high risk of infection compared to other grazing systems ('Tethered : other', OR = 12.00, p = <0.001; 'Communal : Tethered', OR = 0.10, p = <0.001) and the odds ratio of infection in tethered cattle

compared to communal and tethered cattle was 17.33 ('Tethered : Communal and tethered'; p =<0.001).

These high, extremely significant risk levels in tethered cattle may be due to the small number of tethered cattle in the study. This would lead to the risk of the group being easily influenced by randomly higher proportion of infected cattle. Another explanation, however, is that infected cattle are grazed while tethered, allowing the owner to monitor animals more easily than if they were allowed to graze communally.

The odds ratio of 'communal and tethered: other' was 0.52 (p = 0.021) and for 'communal and tethered: communal' was 0.56 (p = 0.044). This indicates that although the risk of infection in animals grazed solely while tethered, there is some protective effect to animals grazing while tethered some of the time.

Grazing System	n	Number of TBR-PCR positives	Pairing	p - value	Odds ratio
Communal	1357	39	Communal : Other	0.377	1.26 (0.76 – 2.09)
Communal and Tethered	1048	17	Communal and Tethered : Other	0.021*	0.52 (0.29 – 0.91)*
Not grazed	3	0	Tethered : Other	0.0001[F]*	12.00 (5.24 – 27.50)*
Tethered	36	8	Tethered : Communal and tethered	0.000*	17.33 (6.90 – 43.50)
			Communal: Tethered	0.0001[F]*	0.10 (0.04 – 0.24)*
			Communal and tethered : communal	0.044*	0.56 (0.31 – 0.99)*
Total	2444	64			

Table 20. Odds ratios between grazing systems for T. brucei s.l. infection.

Fig. 40 shows the higher proportion of AAT infected animals that are managed using the tethered grazing system.

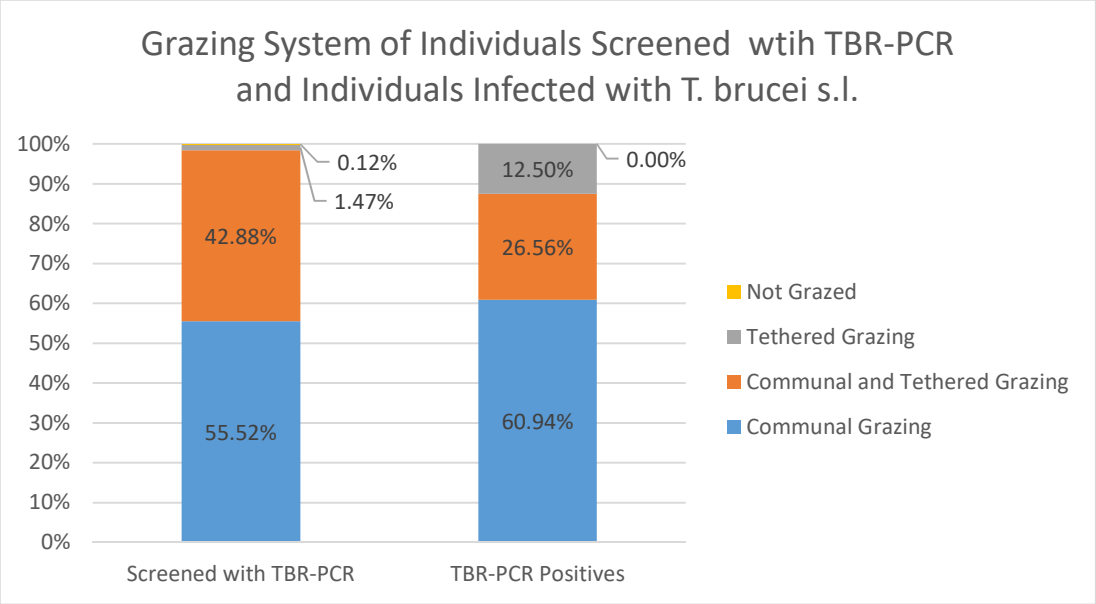

Figure 40. The proportion of animals screened with TBR-PCR and animals infected with T. brucei s.l. in each grazing system.

4. Discussion

The prevalence of *T. brucei* s.l. infection was low across the study area and trypanosomiasis control activities were uncommon. The cattle of the area were predominantly indigenous Zebu breeds, body condition scores were good overall and the communal and tethered grazing was widespread. If *T. brucei* s.l. prevalence is used as a surrogate measure for the overall prevalence of infection by any form of *Trypanosoma* then the area can be said to be stable, with low prevalence of AAT.

4.1. Trypanosomiasis control practices

Despite the endemicity of *G. f. fuscipes* throughout the study area (Aksoy et al, 2014) and low usage of tsetse control methods by farmers, transmission of AAT remains low. The lack of pyrethroid use for spraying of cattle and of trypanocidal drug administration indicates a perception of the low risk posed by trypanosomiasis in this area

The questionnaire responses regarding what drives cattle spraying largely referred to prevention of tick borne diseases rather than a need for tsetse control. This is borne out by the insecticides products used by farmers for spraying their cattle: farmers are reported to spray their cattle with insecticides in 58.33% of sample villages; only 13.89% of sample villages reported farmers spraying with pyrethroid products whereas 44.44% reported spraying with acaricide products effective for reducing control ticks.

Ticks are likely perceived by farmers as a more serious threat to the health of their cattle as tick infestation is visible whereas feeding tsetse are not. Furthermore, in an area where trypanosomiasis is uncommon, it is easy to understand why farmers may target their limited financial resources at limiting tick-borne diseases such as East Coast fever which may be common and can have devastating effects on cattle health.

It was commonly stated in the questionnaire responses that greater accessibility to effective and affordable insecticides and spraying equipment may encourage farmers to spray their cattle more. In this resource poor setting, cost of any animal management practice is important to the extent of its uptake. It is therefore imperative that low-cost control methods that are implementable in this context are developed, particularly as any costs are borne by smallholder farmers themselves in the decentralised Ugandan veterinary system. With an estimated cost of spraying at S$1.72/animal/year when RAP is followed for 25% of the herd, (Muhanguzi et al, 2015) uptake of this practice by farmers in this area would be advantageous as a low cost, environmentally benign and efficacious method of tsetse control should the need arise.

The cost aspect of pyrethroid spraying is also reflected in the number of farmers that spray their herd with products effective only against ticks, not tsetse flies. The use of amitraz products is widespread, Amitix© being a particularly popular product used in 36.11% of villages. Bardosh et al (2013) also found this product to be widely used, with Amitix© holding a 38.2% market share in sales of acaricides. They attributed this to a number of factors, price being among them. They found that limited financial resources and a lack of awareness of the benefits of pyrethroids led farmers to purchase the cheapest product available, this generally being amitraz products. This may be compounded by a lack of information exchange from veterinary drug shop owners to cattle farmers and an incentive to maintain a reputation for having low prices. In this liberalised economy where shopkeepers have limited capital, the product variety stocked is restricted to those in highest demand. Unfortunately, in this setting, this tends to be products ineffective for controlling tsetse, despite shopkeeper knowledge of product effectiveness.

General concerns for the health and productivity of cattle were commonly identified as drivers of spraying cattle. It is therefore important that farmers are equipped with good knowledge of trypanosomiasis control in the event of an AAT outbreak in this area in the future in order to mitigate the impact. A public awareness campaign would be an appropriate means of increasing the knowledge of products and practices for trypanosomiasis control if there are areas in which this is lacking.

Greater contact with veterinary extension workers was identified by one LC1 Chairperson as a factor that may increase spraying and if this were to involve exchange of knowledge regarding the importance of tsetse control, drug shop owners may see the market for pyrethroids goods increase.

Data on other tsetse control methods such as use of traps and targets in the area is unavailable. Successful implementation of these other control methods may contribute to the low transmission rates found in this area.

Kasozo et al (2014) found in a study of treatment of hemo-parasite infections (including AAT) that drug administration is based on clinical signs. Selby et al (2013) also found, with regards to *T. b. rhodesiense*, that the treatment of animals for a disease that they appeared to not be suffering from was perceived as an unnecessary cost. *T. b. rhodesiense* infection tends to be asymptomatic in cattle so there is little incentive for individual farmers to incur this expense. The limited administration of trypanocidal drugs by cattle farmers may be due to overall low prevalence of AAT and therefore a correctly perceived lack of need to purchase and administer these drugs; which can present a significant expense.

This attitude of treatment only being required when animals show symptoms of disease combined with deficiencies in knowledge of proper treatment, may allow prevalence of AAT to increase and a cattle reservoir of asymptomatic *T. b. rhodesiense* to become established in this region.

In the villages where trypanocidal drugs were administered, a number of other drugs were also administered for the treatment of AAT. These drugs were antibiotics lacking in trypanocidal activity. The use of these drugs shows a lack of understanding of AAT at some point in the network of individuals involved in animal healthcare, whether this be on the behalf of veterinary health workers, drug shop owners or the cattle owners themselves. The drugs used further demonstrates the emphasis on prevention of tick borne diseases as they are effective in the treatment of these conditions.

4.2. Trading cattle outside of market system in high risk districts to the south

A number of villages in Lamwo and Kitgum districts reported purchasing cattle from Amach market in Lira and Arapai market in Soroti. Other villages reported purchasing cattle from unidentified locations in Lira district and Oyam district. Cattle in these districts and in these particular markets have been found to be infected with *T. brucei* s.l. and *T. b. rhodesiense* in the past (see section. X.x)

This activity of purchasing cattle from markets and other locations in these high risk districts presents as a constant threat of introducing greater numbers of cattle infected with *T. brucei* s.l. or, more worryingly, *T. b. rhodesiense* into the northern districts where *T. brucei* s.l. prevalence is currently low and *T. b. rhodesiense* is absent. There is a precedent of this mode of expansion of AAT distribution, as seen in the northerly expansion of *T. b. rhodesiense* during the last two decades, (Fèvre et al, 2001; Batchelor et al, 2009) and therefore efforts must be made to prevent the same consequences of cattle movements in the future.

Enforcement of the Ministry of Agriculture (MoA) directive which states that cattle traded in high risk districts must be treated with trypanocide drugs before the issue of transport permits would minimise the effects of these movements. (Wendo, 2002) This measure should, if adhered to, prevent the movement of infected cattle traded at markets, however, this will not be effective with regards to unregulated trade outside the market system, and thus these unofficially traded animals are a greater risk factor for disease spread (Fèvre et al, 2006).

One means of inhibiting the risk attached to trading outside of the market system could be to enforce administration of trypanocide to all cattle introduced to a district from outside the district. As individual cattle owners would be unlikely to voluntarily present their animals for this treatment due to the costs attached, one way in which to reduce avoidance would be to displace the responsibility of this cost from cattle owners to the local veterinary authority. Development of pen-side diagnostics for AAT would allow infected animals to be identified for treatment, reducing the cost of ensuring that animals introduced into the district are free of infection.

4.3. What might upset the currently stable, low transmission system in Northern Uganda

4.3.1. Changing Breed Structure of Herds

The current situation regarding AAT in the northernmost districts of Uganda is stable, with low prevalence. It was found in this study that there is lower risk of infection in the

indigenous Zebu breed than in cattle of other breeds that were screened (OR = 0.40; p = 0.001). There was a significant lower risk in Zebu cattle than in Ankole cattle (OR = 0.36; p = 0.049) and mixed breed animals were at significantly higher risk than non-mixed breed animals (OR = 2.12; p = 0.023). The animals screened were overwhelmingly Zebu breed (87.07%), and smaller proportions were mixed (9.12%), Ankole (3.44%) and exotic breeds (0.37%).

This Zebu-heavy breed distribution is favourable to maintaining the currently low transmission and low prevalence of AAT in the area. With the results of this study adding to previous findings (Anene et al, 1991; Magona et al, 2004) that indicate that certain breeds are more trypanosusceptible, the breed of animals introduced into the area must be a consideration in government restocking programs and the activities of NGOs.

NGOs such as Heifer International provide high yielding, often European, breeds including Holstein-Friesian, Guernsey, Jersey and Ayrshire to farmers in Uganda. (Kabunga, 2015) The higher milk and beef production, as well as draft power output, compared to indigenous cattle are advantageous to farmers but the introduction of these breeds is a double-edged sword if they are more likely to suffer from AAT.

Not only will the number of exotic breeds increase through the activities of these organisations, but it is likely that farmers will preferably breed these high-yielding animals with their indigenous cattle to genetically improve their herds in order to achieve increased milk and meat production. This will lead to a lessening proportion of the cattle being of the more trypanotolerant Zebu breed and an increasing proportion of more vulnerable mixed breeds (Taylor and Authie, 2004).

Furthermore, overall body condition scores of cattle were good, indicating that the carrying capacity for the communally grazed pasture lands of the area is adequate to support the current volume of cattle at this time.

Exotic breeds, which have greater feed and water requirements than the indigenous Zebu, are most often zero grazed, as is encouraged by development agencies. Some farmers, however, due to the labour- and resource-intensive nature of this grazing system revert to more extensive systems (Baltenweck, 2007). If the number of exotic and mixed breed cattle in this area increases and more extensive grazing systems are used, as is seen in this data, then these cattle may not receive high energy and protein intakes that might otherwise offset the deleterious effects of AAT on productivity (Holmes et al, 2000)

4.3.2. Improved Transport links with South Sudan

Further to the risks posed by movement of cattle, the improvement of road and rail infrastructure running through the northern district is a potentially influential development. Upgrading key roads to bitumen and the construction of railways are likely to facilitate the transport of greater numbers of cattle into the area studied and beyond.

4.3.2.1. Gulu-Atiak Nimule Road

The upgrade of the Gulu-Atiak-Nimule Road (see Fig. 41 for the route of this road) from murram, clay-like dirt, to bitumen is a key infrastructure project currently being undertaken. The road runs north from Gulu, the principal city in the Northern Region of Uganda, to Nimule, a South Sudanese border town, through Gulu and Amuru districts.

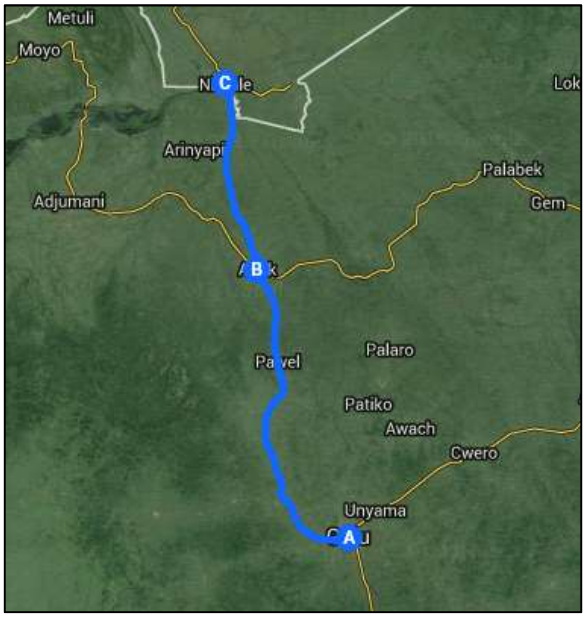

Figure 41. Route of Gulu-Atiak-Nimule Road. Points on route are: Gulu (A), Atiak (B) and Nimule (C)

The Gulu-Nimule road is already important in trade between Uganda and South Sudan, which has increased as the area has become more secure in recent years (The Integrated Fund, 2016). Work commenced on the 74km Gulu-Atiak section of the road in May 2012 and is funded jointly by the Government of Uganda and the World Bank. As of April 2015, the Uganda National Roads Authority stated that work on this section of the road was to be completed by the end of July 2015 (UNRA, 2015). Work on the 37km Atiak-Nimule section of the road commenced in July 2013 and it is reported that the work was due to be complete in May 2016 (The Monitor, 2016).

The improved road surface will reduce transit times and therefore reduce the cost of transporting livestock along this route. This may result in increased numbers of cattle being

introduced from areas south of Gulu into the northernmost districts of Uganda, particularly Amuru, Adjumani and Lamwo which this road runs through or adjacent to. The number of cattle transported into South Sudan is also likely to increase with the reduced transport costs. The porous Ugandan-South Sudanese border may allow the movement of unofficially traded animals, and risk the consequences of disease spread attached to this (see Section 3.7.). This increased movement may lead to increasing numbers of cattle infected with *T. brucei* s.l. and specifically *T. b. rhodesiense* being introduced into areas that are currently stable with regards to ATT and where *T. b. rhodesiense* is currently absent. The increased movement of these animals into South Sudan is particularly worrisome as cross-border cooperation would be required to prevent the possibility of the introduction of *T. b. rhodesiense* into established *T. b. gambiense* foci.

4.3.2.2. Standard Guage Railway

Another large-scale infrastructure project currently underway is construction of a Standard Gauge Railway (SGR) throughout East Africa. This rail network will connect the major cities of the East African Community and included will be a connection between Kampala and Juba via Malaba on the Ugandan-Kenyan border. Fig. 42 shows the route of the entire network including the lines within Uganda. The member states of the East African Community have set the target for the completion of the project as March 2018 ('Roads and Railways: Uganda', 2014)

Figure 42. . Standard Gauge Railway Network (reproduced from The Economist, 2015)

The rail network will allow both the faster and cheaper transport of goods including cattle and will increase the ease with which people can migrate through this vast area.

The same problems associated with increased volumes of cattle movements, as discussed in Section 4.3.2.1,, will be faced with the improved ease of transport made possible by the SGR and it will allow the transportation of animals and the migration of people over even greater distances.

The SGR will allow migration to Kampala and the other cities from areas in which gHAT control measures may currently be interrupted e.g. areas affected by conflict in South Sudan and DRC. Furthermore, as gHAT can be transmitted sexually, as one reported case appears to have been (Rocha et al, 2004), this movement of people may present challenges in urban areas where people are likely to move to for employment, despite the absence of tsetse.

4.3.3. Influx of refugees from South Sudan and DRC

Conflict and human rights abuses are driving refugees and asylum seekers (RAS) from South Sudan and the Democratic Republic of the Congo (DRC) to Uganda. As of 26/4/16 there are 224,748 refugees and asylum seekers (RAS) from South Sudan in Uganda. Of this number, 22,483 arrived prior to 15/12/13 and the remaining 202,265 have arrived 17 months since then. (UNHCR, 2015) As of 29th March 2016, there are 120,761 registered refugees and

asylum seekers from South Sudan in Adjumani district. Figure x shows that the rate at which refugees and asylum seekers are entering Uganda has been increasing since December 2015.

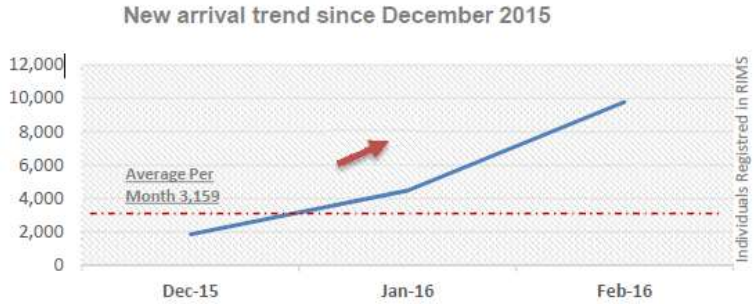

Figure 43. Volume of refugees and asylum seekers arriving in Uganda. RIMS = Refugee Information Management System (reproduced from UNHCR, 2015).

According to UNHCR figures, the majority of refugees currently living in the northern region of Uganda have fled South Sudan but throughout Western Uganda and Kampala a large portion of the refugees present originate from the Democratic Republic of Congo (DRC). As of 1st April 2016, the total number of refugees and asylum seekers in Uganda from all source countries is 525,968. (UNHCR, 2016)

People from a number of countries are fleeing and seeking refuge in Uganda but the significance of people coming from DRC and South Sudan is that these two countries are affected by *T. b. gambiense* and individuals may originate from high transmission areas. Prevalence of *T. b.* gambiense infection may be high. Conflict disrupts surveillance and control programs, and as an area becomes depopulated and the agricultural use of land is interrupted the habitat reverts to a natural state, more suitable for tsetse flies (Berrang-Ford, 2007), as was seen in Soroti district during the 1980s-1990s (Hutchinson et al, 2003).

As well as breakdown of tsetse control causing surges in gHAT incidence, Welburn et al (2016) propose that asymptomatic "silent" carriers of *T. b. gambiense* may lose their tolerance and succumb to infection in times of stress and famine. This may explain the strong link between conflict and HAT cases investigated by Berrang-Ford et al (2011).

Individuals originating from high incidence areas that are subject to stress and poor nutritional conditions may well contribute to an expansion of the *g*HAT focus towards the rHAT focus in Uganda as they move throughout the country. If these individuals are mobile, the sexual transmissibility of *T. b. gambiense* (Rocha et al, 2004) will further increase the potential for spread of gHAT. Resources ought to be made available to provide screening to individuals arriving from high risk areas. This could be carried out by the UNHCR integrated into the current refugee registration process.

In addition to the potential challenges of an expansion of the gHAT distribution posed by individuals arriving in Uganda, the return to people's homes may also have damaging consequences. If individuals bring cattle with them when fleeing their homes and/or acquire cattle while seeking refuge in Uganda, there is a risk that these animals may become carriers of infection. Upon return to their homes these animals may carry this infection and, in the presence of a viable vector, these parasites may be transmitted to other animals/humans. This could lead to expansion of the prevalence of AAT within the cattle population of these areas and in the event that cattle are infected with zoonotic *T. b. rhodesiense,* new rHAT foci may emerge. This is a particularly worrying prospect in areas which, as discussed, may also be affected by gHAT due to interruption of control measures.

4.4. Limitations of methodology

4.4.1. Village level data collection

Any statements regarding the pervasiveness of certain practices assumes homogeneity within villages. For example, in a given village, every farmer may spray their animals with Amitix© and in another village, only one farmer may use this product. With this current method of data collection, the LC1 of each village would both identify farmers in their village as using this product and therefore the data would be identical i.e. a lack of resolution within the data. If this data was collected at the level of the individual farmer, the reality would be represented.

This is not to say, however, that collecting this data at village level does not provide useful information regarding cattle management in the area, only that more precise, higher resolution data could be collected using farmer-level interviews.

Interviews would avoid problems of misinterpretation of questions and of answers lacking in detail that arise with self-completed questionnaires. These difficulties are particularly prominent in a context where the ability to communicate in written English is not ubiquitous. Collecting data by means of interviews would necessitate that field teams are able to communicate competently in local languages.

During the data and specimen collection visits to villages, a member of the DVO's staff literate in both English and the local language was always present and they would be able to conduct the interviews while translating any queries to a member of the field team.

Conducting interviews would be easily achievable as cattle owners would be identified by the LC1 and, moreover, they tend to be present during specimen collection from their cattle. In order to collect the same information, the interview would not be overly time consuming

compared to questionnaires and would have the advantage of collecting more accurate, precise data.

4.4.2. The concept of the village and its implications for inclusion of animals in the study

In a number of villages, the case arose where a farmer, resident to that village, grazed their cattle some distance away from the boundaries of the village itself. Animals grazed in a different geographical location to the rest of the village herd may be exposed to a different level of tsetse challenge and to animals in which prevalence of trypanosome infection is higher or lower.

It could be argued that, as the epidemiological unit for the study is the village, which is a defined geographical area, animals grazed outside of this area should not be included in this village. The geographical location in which animals live should be given primacy over the village of which their owner is resident; however, animals may be grazed in a number of areas and it may not be feasible to determine which animals should and should not be included. It is possible, however, to capture data regarding cattle movements using wearable GPS devices and remotely sensed satellite images Handcock et al, 2009). Solar-powered tags weighing less than 40g that allow continuous monitoring of livestock position are in development (Panckhurst et al, 2015). The information gained from such devices may inform decisions as to whether or not animals ought to be included in the 'village' in future studies.

4.4.3. Cattle trade data

The data obtained through the collection method in this study provides information of which locations were receiving cattle from which markets and districts. This information is useful in determining areas at risk of introducing infected cattle from high risk districts. An improvement on this information could be made through obtaining information on the volumes of cattle traded. This information could be obtained, as suggested previously, by interviewing individual cattle farmers rather than administering questionnaires to LC1 Chairpersons.

Records of cattle movement permits could also be obtained from district veterinary offices. This would allow corroboration of farmers accounts of cattle trade and furthermore, any discrepancy between the accounts would indicate the degree to which cattle without license are introduced into each district.

4.5. Future Research

Following from the findings of this study and the discussion of how the current circumstances may change in the future, there are a number of avenues of future enquiry that will provide greater depth of knowledge of the current situation and allow the for monitoring of developments in the future.

Continued surveillance of AAT in cattle

Continued surveillance of AAT prevalence in the cattle of this area would allow anticipation of the need for future intervention, should the current situation worsen. The findings of this study provide prevalence data for a point in time whereas ongoing surveillance would allow for the identification of trends and of the expansion or retraction of disease distributions.

Research into attitudes and awareness of trypanosomiasis control

It would be beneficial to study, in greater depth, the attitudes towards and awareness of trypanosomiasis control in farmers in this area. Prevalence of AAT is currently low but if there were to be an outbreak in this area, knowledge of this would allow for efficient and targeted sensitisation campaigns. Perceptions of trypanosomiasis control measures (including insecticide spraying and trypanocide administration) knowledge of the disease processes and how they relate to human disease, and attitudes towards support provided by the state would be a useful insight for informing policy and interventions.

The research carried out by Bardosh et al (2013) provided interesting insights into the drivers of the insecticide market and an expansion of this into the northernmost districts of Uganda would provide useful knowledge. A sampling strategy identical to the one used in the current study for determining the study villages could be used. Interviews with cattle owners in sample villages would provide a detailed and nuanced view of attitudes and knowledge across the study area whereas interviews with key figures i.e. District Veterinary Officers, veterinary drug shop owners and others involved in the control of trypanosomiasis would give insight into the management of trypanosomiasis from a range of perspectives and representing a range of interests. This method of data collection could be integrated into future surveillance programs and could use the same village-level sample, as was done in study.

Surveillance of trypanosomiasis in refugees and asylum seekers from gHAT-affected areas

As discussed, there is a risk of humans carrying *T. b. gambiense* seeking refuge in Uganda. Refugees in general often have special healthcare requirements and are confronted by a range of health issues not faced by the general population but in the context of Uganda with

regards to HAT, surveillance in these at-risk groups is of importance in monitoring any developments in the Ugandan gHAT/rHAT situation. Not only would this enable the anticipation of an expansion of the gHAT focus but would also ensure that these people receive the care that they require. Surveillance of people arriving in Uganda could be integrated into the current refugee registration systems, as the formal structure through which many individuals already pass is pre-existing and well defined.

Investigating volume of cattle trade

Cattle movements have long been implicated in the expansion of trypanosomiasis and, as found in this study, cattle are being introduced from areas to the south into the northernmost districts. In order to assess the scale of this threat to the stability of these districts and to target any required intervention, the volume of these cattle movements must be known. The findings of this study provided information on the locations from which cattle are purchased but no information on the volume of trade was gathered. Attempting to quantify the extent of the undocumented cattle trade would provide valuable knowledge of this high-risk activity but gathering accurate data would present difficulties.

Surveillance of people and cattle transported by the improved infrastructure network

As the road and rail projects currently underway in Uganda and in the wider East African region are completed, the challenge that they pose to management of trypanosomiasis would need to be investigated. Information regarding the volume of people and animals moving through these means, their origins and destinations, and the prevalence of trypanosomal infection within these groups would allow for this potentially dramatic shift in the region to be anticipated. Data collection and screening could be conducted at train stations throughout the rail network but this would be much more difficult on the road system.

4.6. Conclusion

Six districts across the Northern region of Uganda were assessed for the presence of the cattle parasite T. brucei s.l. Specimens were collected and screened from 39 villages across the area in accordance to a two stage cluster sampling protocol in which the primary sampling unit was the village and the secondary was the individual animals within included villages, of which 25% were screened.

The prevalence of T. brucei s.l. infection in the cattle of the studied area was 2.61%, and it is inferred from this that the prevalence of cattle trypanosomiasis, in the broader sense, is also low. If body condition score can be considered as an indicator of lack of disease in general, and of high quantity and quality forage in this largely communal grazing system, then the

high condition scores of the animals and low trypanosomiasis prevalence across the area paint a positive picture of the health of the cattle and the carrying capacity of the land.

This low level of disease was found despite low usage of trypanocidal drug treatment and spraying of cattle with pyrethroid insecticide as means of tsetse control. The use of insecticides effective against ticks, and the responses to survey questions regarding drivers of insecticide spraying, indicate that tick challenge and tick-borne disease are far more prominent concerns to the cattle owners of this area. Herds largely consis of indigenous Zebu cattle, but there are smaller proportions of exotic breeds and cross breeds present in these herds. The pervasiveness of these trypanotolerant animals may contribute to the low prevalence of trypanosomiasis.

The degree to which cattle are used for traction varies widely across the study area, potentially indicating differences in the implementation of mixed-crop livestock production. In districts where the use of adult males for traction is common, high prevalence of trypanosomiasis in cattle would significantly impact on the draft power output and the capacity for agricultural production. Furthermore, herds across the whole study area largely are female-biased, indicating that milk production is of great economic importance. Preservation of the low trypanosomiasis prevalence in the cattle of this area is therefore crucial to safeguarding the output of rural economy.

The current stability of this area faces a number of potential threats, from increased cattle movements facilitated by transport infrastructure improvement, introduction of cattle from high prevalence areas through trade and restocking programs, and the keeping of an increasing proportion of high yielding, but trypanosusceptible, cattle breeds.

The ever-looming threat of sympatric gHAT and rHAT foci will also be made more potent by these developments. As transport improvements have the potential to facilitate increased movement of cattle, so too might they lead to increase movement of people. This may, along with the current humanitarian crisis in the DRC and South Sudan, lead to individuals potentially infected with T. b gambiense migrating to areas currently unaffected by this parasite. With potential expansion of the cattle reservoir of T. b. rhodesiense, through cattle trade and refugees returning to their homes, and the migration of individuals from T. b. gambiense-affected regions, the discrete nature of the two disease foci might not last.

These threats to the status quo of trypanosomiasis in Uganda must be recognised and developments must be monitored as there is potential for a situation to evolve which has

serious negative outcomes for the health and economic security of the population of rural northern Uganda.

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